Heterozygous germline BLM mutations increase susceptibility to asbestos and mesothelioma

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Rare biallelic BLM gene mutations cause Bloom syndrome. Whether BLM heterozygous germline BLM+/- mutations cause human cancer remains unclear. We sequenced the germline DNA of 155 mesothelioma patients (33 familial and 122 sporadic). We found 2 deleterious germline BLM+/- mutations within 2 of 33 families with multiple cases of mesothelioma, one from Turkey (c.569 570del; p.R191Kfs*4) and one from the United States (c.968A>G; p.K323R). Some of the relatives who inherited these mutations developed mesothelioma, while none with nonmutated BLM were affected. Furthermore, among 122 patients with sporadic mesothelioma treated at the US National Cancer Institute, 5 carried pathogenic germline BLM+/- mutations. Therefore, 7 of 155 apparently unrelated mesothelioma patients carried BLM+/- mutations, significantly higher (P = 6.7E-10) than the expected frequency in a general, unrelated population from the gnomAD database, and 2 of 7 carried the same missense pathogenic mutation c.968A>G (P = 0.0017 given a 0.00039 allele frequency). Experiments in primary mesothelial cells from BLM+/- mice and in primary human mesothelial cells in which we silenced BLM revealed that reduced BLM levels promote genomic instability while protecting from cell death and promoted TNF-α release. BLM+/- mice injected intraperitoneally with asbestos had higher levels of proinflammatory M1 macrophages and of TNF-α, IL-1β, IL-3, IL-10, and IL-12 in the peritoneal lavage, findings linked to asbestos carcinogenesis. BLM+/- mice exposed to asbestos had a significantly shorter survival and higher incidence of mesothelioma compared to controls. We propose that germline BLM+/- mutations increase the susceptibility to asbestos carcinogenesis, enhancing the risk of developing mesothelioma.

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increased susceptibility to asbestos-induced mesothelioma in mice (9, 10), evidence of GxE. Reduced BAP1 levels impair DNA repair (11) as well as different forms of cell death (3, 12) and induce metabolic alterations (13–15) that together favor cancer development and growth.

Recent studies revealed that mesothelioma may also develop among carriers of germline mutations of additional tumor-suppressor genes that cause well-defined cancer syndromes, including MLH1 and MLH3 (Lynch syndrome), TP53 (Li-Fraumeni syndrome), and BRCAl-2 (Breast and Ovarian Cancer syndrome) (16, 17). When all germline mutations are combined, it has been estimated that about 12% of mesotheliomas occur in carriers of heterozygous germline mutations of BAP1, the most frequent mutation among patients with mesothelioma, or of other tumor suppressors. Some of these mutations may sensitize the host to asbestos carcinogenesis, according to a GxE scenario (17). Thus, presently, mesothelioma is considered an ideal model to study GxE in cancer (17). As part of the Healthy Nevada Project (HNP), we are studying GxE in northern Nevada, a region with an unusually high risk of exposure to carcinogenic minerals and arsenic, which may be related to the high cancer rates in this region (18). We are investigating genetic variants that may increase cancer risk upon exposure to carcinogens to implement preventive strategies.

Biallelic mutations of the Bloom syndrome gene (BLM) cause Bloom syndrome, an autosomal-recessive tumor predisposition syndrome characterized by pre- and postnatal growth deficiency, photosensitivity, type 2 diabetes, and greatly increased risk of developing various types of cancers. BLM is a RecQ helicase enzyme that modulates DNA replication and repair of DNA damage by homologous recombination (19). In patients affected by Bloom syndrome, the absence of the BLM protein causes chromosomal instability, increased number of sister chromatid exchanges, and increased numbers of micronuclei (20–22). In addition, BLM is required for p53-mediated apoptosis (23), a process critical to eliminate cells that have accumulated DNA damage. Impaired DNA repair together with altered apoptosis resulted in increased cancer incidence (17, 24). Of course, inactivating germline BLM heterozygous (BLM+/–) mutations are much more common than biallelic BLM (BLM−/−) mutations, with an estimated frequency in the general population of 1 in 900 based on data from the Exome Aggregation Consortium (25). BLM+/– mutation carriers do not show an obvious phenotype; however, some studies have suggested that carriers of these mutations may have an increased cancer risk (17, 24). Mice carrying BLM+/– mutations are prone to develop a higher rate of malignancies in the presence of contributing factors, such as concurrent heterozygous mutations of the adenomatous polyposis coli (Apc) gene, or upon infection with murine leukemia virus (26). However, in studies in which Blm+/– mice were crossed with tuberous sclerosis 1-deficient (Tsc1+/–) mice that are predisposed to renal cystadenomas and carcinomas, Wilson et al. found that Tsc1+/–/Blm+/– mice did not show significantly more renal cell carcinomas compared with Tsc1+/–/BlmWT mice (27). In humans, a large study involving 1,244 patients with colon cancer and 1,839 controls of Ashkenazi Jewish ancestry, in which BLM+/– frequency is as high as 1 in 100 individuals (28), suggested that carriers of germline BLM+/– mutations might have a twofold increase in colorectal cancer (CRC) (29). A smaller study did not confirm these results, but reported a trend of increasing incidence of adenomas—premalignant lesions—among BLM+/– mutation carriers (30). In addition, BLM+/– mutations were found overrepresented among early-onset (<45 y old) CRC patients (25). Other studies associated BLM+/– mutations to an increased risk of breast (31, 32) and prostate cancer (33), but the low power of these studies hampered definite conclusions. In summary, it appears possible that BLM+/– mutations may increase cancer risk in the presence of contributing factors.

Results

Identification of Two Families with High Incidence of Mesothelioma and BLM Mutations. We used whole-genome sequencing (WGS) to test germline DNA of 10 patients with mesothelioma in 4 Turkish families with multiple cases of mesothelioma. Two patients from the same family (mother and son) carried the heterozygous BLM mutation chr1:19293067_19293068, c.569_570delAG; p.R191Kfs*4 (Fig. 1A and Table 1). This mutation leads to a frameshift, which creates a truncated BLM protein of 193 aa. This mutation has not been reported in gnomAD or 1000 Genomes and is not listed in ClinVar, evidence that it is very rare. Sanger sequencing confirmed the mutation in these two patients and revealed the presence of the same mutation in a currently healthy 15-y-old brother, while his healthy father and sister had wild-type (WT) BLM (Fig. 1A and SI Appendix, Fig. S1A and Table S1).

The findings in the Turkish family prompted us to test the germline of 29 mesothelioma patients, 28 from the United States and 1 from Turkey, with a family history of mesothelioma in 2 or more family members, who had tested negative for BAP1 mutations. Sanger sequencing of the entire BLM gene revealed that 1 of 29, a 57-y-old US female (family 2), carried a rare heterozygous missense mutation c.968A>G; p.K323R of the BLM gene (rs146504061, allele frequency in the general population, gnomAD T = 0.00039; Fig. 1B, Table 1, and SI Appendix, Fig. S1B). The sequencing of germline DNA extracted from four of her siblings revealed that two of them carried the same mutation: 1) her brother, who had previously developed melanoma and subsequently developed mesothelioma at age 67 y; and 2) a 59-y-old sister who is presently tumor-free (Fig. 1B). The father and the mother in the same family had died of cancer—melanoma and uterine carcinoma, respectively—and their DNA were not available for testing (Fig. 1B).

In silico analyses of DNA sequence variants (mutationtaster.org, genetics.bwh.harvard.edu) and Combined Annotation Dependent Depletion (CADD) scores indicated that both BLM mutations are predicted to be pathogenic (Table 1). The CADD scores were remarkably high: 33.0 for the Turkish family (family 1) and 24.0 for the US family (family 2). Immunostaining of tumor biopsies revealed the absence of BLM nuclear staining in malignant mesothelioma (MM) cells of both Turkish patients (Fig. 1C and SI Appendix, Fig. S1C), evidence of biallelic BLM inactivation in the tumor cells. Tumor biopsies for the US families were not available.

Prevalence of Germline BLM Mutations in Mesothelioma. We tested the hypothesis that BLM mutations might be unusually frequent among patients with mesothelioma in a cohort of 122 patients with pleural and peritoneal mesothelioma treated at the US National Cancer Institute (NCI) for whom germline DNA was available. WES revealed that 5 of 122 patients, 2 with pleural mesothelioma and 3 with peritoneal mesothelioma, carried pathogenic BLM mutations, with a CADD score >20 (Table 1). One of these mutations, c.968A>G, was identical to the mutation found in the US family (family 2); however, these patients do not appear to be related. No other pathogenic mutations were found in these patients that could be linked to an increased risk of developing mesothelioma. Three of these five patients had a family history of cancer, and three of five self-reported a history of asbestos exposure (Table 1). All five patients are experiencing a significantly prolonged survival, a common trait among patients developing mesothelioma in a setting of germline mutations (34). Moreover, all these mesotheliomas were of the epithelial type, like most mesotheliomas developing in carriers of germline mutations.

In summary, 7 of 155 unrelated patients with mesothelioma carried germline pathogenic BLM+/– mutations (SI Appendix, Fig. S1D), a highly significant incidence (P = 6.7E-10) compared to the expected frequency of pathogenic BLM mutations in the general population of 1 in 900 (25), and as high as the 1-in-100 incidence among people of Ashkenazi Jewish ancestry, who carry
a specific set of mutations (29) that were not found in any of our patients, as none of them were of Ashkenazi Jewish ancestry. Moreover, two of seven apparently unrelated BLM-mutated mesothelioma patients carried the same missense mutation c.968A>G, statistically significant (P = 0.0017) given an allele frequency of 0.00039. In summary, the probability of finding heterozygous germline BL M mutations is much greater among mesothelioma patients than in the general population.

In parallel, we analyzed 29,553 individuals from the HNP (18) with whole-exome sequencing (WES) for loss-of-function BL M mutations. Loss-of-function variants were predicted from Loss Of Function Transcript Effect Estimator (LOFTEE) on the Matched Annotation from National Center for Biotechnology Information and European Molecular Biology Laboratory transcript, all with a CADD score >20 or high-confidence LOFTEE prediction. There were 28 BL M heterozygous loss-of-function variants in this dataset: 12 frame-shift variants, 2 splice acceptor variants, 5 splice donor variants, and 9 stop gained variants (SI Appendix, Table S2). There were 80 individuals associated with these variants—some of them may be relatives; this information was not available at this time—and, of these, there were 74 with matched electronic healthcare records. Within the group of 74

![Image](https://example.com/image.png)

**Fig. 1.** Germline heterozygous BL M mutations found in two different families with history of mesothelioma. (A and B) Pedigree and tumor spectrum of family 1 (A) and family 2 (B; Table 1). Carriers of germline BL M mutations are indicated by red stars; BL M WT is marked by green stars. BCC, basal cell carcinoma. Age at diagnosis or BL M testing is indicated in parentheses. Black arrows indicate the probands. (C) BL M immunostaining in tumor tissue sample from family 1 (female). Mesothelioma, transitional type, BL M lost in tumor cells; 5-μm section stained with H&E (i); and BL M 1:350 (ii), photomicrograph at 400x. (Scale bar: 50 μm.) The antibody used for staining recognizes an epitope on the BL M protein at aa 319 to 462; since the truncated form of BL M carried in family 1 is at aa 193, the staining reflects only the presence of the WT protein (SI Appendix, Fig. S1).

![Table 1](https://example.com/table.png)

**Table 1. Summary of genetic and demographic data of the identified MM patients carrying heterozygous BL M germline mutations**

| ID  | Sex | Asbestos exposure | Cancer type (age of diagnosis, y) | MM histology | Survival | BL M CDS SNV position | Amino acid change | Frequency† | CADD | Cancers in family (first and second) |
|-----|-----|------------------|-------------------------------|--------------|---------|----------------------|------------------|-----------|-----|-------------------------------------|
| 1–1 | F   | Yes              | PleMM (62) (age 51)           | Epi          | Unknown | c.569_570del         | R191Kfs*4        | N/A       | 33  | 1 (son)                              |
| 1–2 | M   | Yes              | PleMM (42) (age 39)           | Epi          | Unknown | c.569_570del         | R191Kfs*4        | N/A       | 33  | 1 (mother)                           |
| 2–1 | F   | No               | Ple, PerMM (57) (age 62)      | Biph         | 2 mo    | c.968A > G           | K323R            | 3.86E-04  | 24  | 10 (B, PA, PC)                      |
| 2–2 | M   | No               | PleMM (60), PerMM (67) (age 60) | Epi     | 2 y     | c.968A > G           | K323R            | 3.86E-04  | 24  | 10 (S, PA, PC)                      |
| 3*  | F   | No               | PerMM (62) (age 64)           | Epi          | 11 y    | c.43C > T            | R15C             | 2.44E-04  | 32  | 3 (both parents, PGM)               |
| 4   | M   | Yes              | PleMM (53) (age 57)           | Epi          | 1.7 y   | c.11T > C            | V4A              | 1.12E-03  | 23.4| 1 (S, MU)                           |
| 5*  | M   | Yes              | PerMM (54), thyroid Ca (age 54) | Epi     | 6 y     | c.968A > G           | K323R            | 3.86E-04  | 24  | 2 (S, MU)                           |
| 6   | M   | Yes              | PerMM (61) (age 63)           | Epi          | 3.1 y   | c.2237C > T          | A746V            | 4.62E-05  | 24.6| 1 (MGM)                             |
| 7   | M   | No               | PleMM (64) (age 67)           | Epi          | 4.6 y   | c.2119C > T          | P7075            | 1.66E-03  | 22.4| 0                                   |

PleMM, pleural malignant mesothelioma; PerMM, peritoneal malignant mesothelioma; Epi, epithelial; Biph, biphasic; B, brother; S, sister; PA, paternal aunt; PC, paternal cousin; MU, maternal uncle; PGM, paternal grandmother; MGM, maternal grandmother. Survival calculated from date of diagnosis.

†RefSeq ID for BL M variants: NM_000057.

‡gnomAD frequency: not applicable as of May 2020, not reported in gnomAD.

§Patients who are alive as of August 2020. Family 1 is from Turkey; all others are from the United States.
Reduced BLM Levels Increase Genome Instability while Protecting Primary Mesothelial Cells from Asbestos-Induced Apoptosis and Promoting TNF-α Release from Macrophages. The powerful activity of heterozygous germline BAP1 mutations to facilitate asbestos-induced transformation and cause mesothelioma has been linked to the alterations in the mechanisms that regulate DNA repair and cell death caused by decreased BAP1 protein levels (8). Since BLM is also involved in DNA repair and chromosome segregation (20), as well as apoptosis (23), we investigated if similar mechanisms might regulate G0Xe in carriers of heterozygous germline BLM mutations, making them more susceptible to asbestos carcinogenesis. Increased genomic instability, quantified as higher number of micronuclei that are a consequence of chromosome breakage, was detected in mouse fibroblast cultures heterozygous for Blm (26). We investigated if Blm heterozygosity increases genomic instability in murine mesothelial cells exposed to asbestos. We found that micronuclei occur at a higher frequency in Blm+/− mesothelial cells compared to WT [phosphate-buffered saline (PBS) solution, WT, 2.37 ± 0.88%; Blm−/−, 4.82 ± 2.45%; P < 0.05]; notably, in Blm+/− murine mesothelial cells exposed to crocidolite, we found an even more remarkable increase in the number of micronuclei compared to WT cells (crocidolite, WT, 3.43 ± 1.77%; Blm+/−, 10.68 ± 7.24; P < 0.001; Fig. 2 A and B).

Phosphorylation of histone H2A.X (γ-H2A.X) is an early cellular response to the induction of DNA double-strand breaks (DSBs), and can be measured to assess DNA damage and repair. Phosphorylation of H2A.X was shown to be delayed in BLM-deficient cells (35). We investigated if BLM facilitates the phosphorylation of H2A.X and the recruitment of DNA repair factors in cells exposed to asbestos. These experiments could not be performed in primary Blm+/− murine mesothelial cells because only a small number of these cells can be obtained from mice. Therefore, we used primary human mesothelial (HM) cells obtained from non-malignant pleural fluids, and BLM was silenced using four individual siRNAs targeting BLM (siBLM) or their pool (siBLM-pool). The reduced expression levels of the BLM mRNA and protein were compared to a control (scrambled) siRNA (SI Appendix, Fig. S2 A and B). In BLM-silenced HM cells exposed to crocidolite asbestos, we detected reduced γ-H2A.X levels compared to control (Fig. 2C). Moreover, in a time-course experiment mimicking the progression of DNA repair over time after exposure to asbestos, we observed a delay (~30 min) in the phosphorylation of H2A.X in BLM-silenced cells (Fig. 2D), consistent with what was previously reported (35). BLM cooperates with p53 to induce apoptosis (23); however, despite the increased genomic instability and delay in DNA repair, p53 levels did not appear to change in BLM-silenced HM cells, even upon exposure to crocidolite (Fig. 2 C and D).

Moreover, silencing of BLM induced a significant increase in the viability of HM cells upon crocidolite exposure, expressed as percentage of viable cells compared with vehicle-exposed cells (scrambled, 24.2 ± 5.3%; siBLM-pool, 44.1 ± 10.9%; P < 0.01; Fig. 2E). We linked this effect to reduced cleaved caspase-3 levels, evidence of reduced apoptosis in these cells (Fig. 2F). These findings were reproduced in three different primary HM cells. Therefore, reduced BLM protein levels impaired apoptosis in HM cells exposed to crocidolite, providing a mechanistic rationale for the increased cell viability (Fig. 2E) beside the accumulation of DNA defects (Fig. 2 A–D). We also tested whether reduced BLM expression influenced the survival of three mesothelioma cell lines—Mill, HMESO, and H2596—harboring WT BLM gene, as assessed by Sanger sequencing. The cells were transfected with siBLM, and the reduction in BLM protein levels was assessed compared to control scrambled siRNAs (SI Appendix, Fig. S2C).

Silencing of BLM did not influence cell growth (SI Appendix, Fig. S2D) but partially protected mesothelioma cells from apoptosis, regardless of the cell line or the stimulus used: H2O2 (SI Appendix, Fig. S2E) or ceramide (SI Appendix, Fig. S2F).

We next tested whether BLM silencing influences in vitro transformation of HM cells. HM cells in tissue culture were exposed to crocidolite in media containing TNF-α, a well-established assay to measure asbestos-mediated transformation, as, under these conditions, HM cells become morphologically transformed and form tridimensional foci (3, 36, 37). Surprisingly, we found no significant differences in the number of foci between BLM-silenced cells compared to scrambled control cells (scrambled, 69.3 ± 11.4; siBLM-pool, 63.5 ± 7.0; P = 0.425; Fig. 2G), suggesting that reduced BLM levels are insufficient per se to increase HM cell susceptibility to asbestos.

Asbestos carcinogenesis is largely an indirect process causally linked to asbestos-induced chronic inflammation and to the release of TNF-α and of other cytokines from macrophages, which accumulate at the sites of asbestos deposition (36, 38–42). We wondered whether, in vitro, by adding TNF-α, we artificially leveled any differences between WT BLM and BLM mutant cells. We tested the hypothesis that reduced BLM levels might increase susceptibility to asbestos only in the presence of inflammation. Silencing of BLM protected human THP-1 cells differentiated into macrophages from crocidolite-induced apoptosis (Fig. 2H). Moreover, BLM silencing caused increased release of TNF-α, both in vehicle-treated macrophages (scrambled, 2,125.3 ± 132.6 pg/mL; siBLM-pool, 2,968.5 ± 215.2 pg/mL; P < 0.01) and in macrophages exposed to crocidolite (scrambled, 24,30.7 ± 145.4 pg/mL; siBLM-pool, 2,737.8 ± 87.8; P < 0.05; Fig. 2I).

Experiments in Blm Heterozygous Mice Support Increased Susceptibility to Asbestos. We tested whether heterozygous Blm deletion might make mice more susceptible to asbestos carcinogenesis. Blm+/− mice and WT littermates were injected with 5 mg of crocidolite asbestos [10 intraperitoneal (i.p.) injections of 0.5 mg each time, twice a week for 5 wk; SI Appendix, Fig. S3A]. One week after the last injection, we collected peritoneal lavage to assess the inflammatory response to asbestos, a finding that previous studies linked to asbestos carcinogenesis (37). We observed a significant increase in the percentage of M1 macrophages (CD45+;F4/80+, CD86+, CD206−) in Blm+/− mice compared to WT littermates (P = 0.015; Fig. 3 A and B). We also observed a nonsignificant reduction of M0 macrophages (P = 0.0848; CD45+, F4/80+, CD86−, CD206−; SI Appendix, Fig. S3B). No changes were observed in other macrophage subtypes and neutrophils (SI Appendix, Fig. S3 C–E). Since M1 macrophages are proinflammatory, we tested the profiles of cytokines and chemokines present in the peritoneal lavages of Blm+/− mice compared to WT littermates. The levels of TNF-α, IL-1β, IL-3, IL-10, and IL-12(p70) were significantly higher in Blm+/− mice exposed to asbestos compared to WT littermates (Fig. 3 C–G). These cytokines, especially TNF-α and IL-1β, have been linked to asbestos carcinogenesis and play a critical role in mesothelioma development (36, 38–42). No significant differences were observed in other cytokines and chemokines (SI Appendix, Fig. S4).

In a parallel experiment, we tested the long-term effects of crocidolite exposure in Blm+/− mice compared to WT littermates injected i.p. with 5 mg of crocidolite (10 i.p. injections of 0.5 mg each, once a week for 10 wk; SI Appendix, Fig. S5A). Serum samples were collected 1 wk and 3 mo after the last i.p. injection, and the cytokines and chemokines analyzed. Compared to WT, Blm+/− mice showed a significant increase in the serum levels of IFN-γ and IL-6 at 1 wk (SI Appendix, Fig. S5 B and C) and 3-mo time points (SI Appendix, Fig. S5 D and E). No significant differences were observed in the serum levels of TNF-α, IL-1β, IL-3, IL-10, and IL-12 (SI Appendix, Figs. S6 and S7). These results indicated that
Fig. 2. Reduced BLM levels promote genome instability while protecting from crocidolite-induced cell death and promoting TNF-α release from macrophages. (A and B) Murine mesothelial cells were isolated from Blm<sup>−/−</sup> mice and WT littermates, and chromosomal instability was determined as micronuclei frequency at interphase. (A) Cells were treated with 5 μg/cm² crocidolite for 8 h or left untreated (PBS); 48 h later, the number of micronuclei (indicated by white arrows) was determined by DAPI staining. (Scale bars, 5 μm.) (B) Percentage of interphase cells with micronuclei in ≥140 cells counted per treatment from n = 2 WT control and n = 4 Blm<sup>−/−</sup> mice in n = 2 independent experiments; data are shown as mean ± SD. (C–G) HM cell cultures were transfected with control siRNA (scrambled) or a pool of siRNAs-BLM (siBLM-pool) for 48 h. (C and D) Western blot comparing γ-H2A.X levels. (C) DNA damage in HM cells exposed to 5 μg/cm² crocidolite for 8 or 24 h (+) or left untreated (−). (D) Kinetics of DNA repair in HM cells treated with 5 μg/cm² crocidolite for 24 h and harvested after the indicated time of DNA rejoin. In C and D, decimals indicate the amounts of γ-H2A.X normalized to H2A.X, calculated as the ratio of the arbitrary optical densitometry units (measured using ImageJ) of γ-H2A.X bands normalized to H2A.X bands. (E) Viability of HM cells treated with 5 μg/cm² crocidolite for 24 h; data shown as mean ± SD (n = 6). (F) Cleaved caspase-3 levels in HM cells treated with 5 μg/cm² crocidolite for 24 h. (G) Foci formation after 4 wk of culture in HM cells exposed to 5 μg/cm² crocidolite for 24 h or left untreated (−). (H and I) THP-1 cells were treated with 20 μM TPA for 24 h to induce monocyte differentiation into macrophages; subsequently, cells were transfected with control scrambled siRNA or siBLM-pool for 24 h and then treated with 5 μg/cm² crocidolite for 24 h. (H) Cleaved caspase-3 levels and transmembrane TNF-α levels. (I) TNF-α levels in the culture media measured by ELISA; results are shown as mean ± SD (n = 3). The BLM antibody (BLM predicted molecular weight, 159 kDa) recognizes a double band at ~150 to 170 kDa (indicated by the arrow in D). In F and H, decimals indicate densitometry units of cleaved caspase-3 levels or transmembrane TNF-α normalized to α-tubulin. P values were calculated using a two-tailed unpaired Welch’s t test (*P < 0.05; **P < 0.01).
Germline Blm<sup>+/−</sup> influenced the peritoneal inflammatory response upon asbestos exposure. Blm<sup>+/−</sup> mice and WT littermates were monitored for a 16-mo follow-up period after the last i.p. injection of crocidolite asbestos (SI Appendix, Fig. S5A). The incidence of mesothelioma was higher in Blm<sup>+/−</sup> mice (21 of 25 mice developed MM, 84.0%; P = 0.0453) compared with WT littermates (13 of 23 mice developed MM, 56.5%; Fig. 3H). Moreover, Blm<sup>+/−</sup> mice had a significantly shorter mesothelioma-free survival (median survival, 334 d; P = 0.0264) compared to WT littermates (median survival, 362 d; Fig. 3I). Chronic inflammation leading to intestinal occlusion was the cause of death in 3 of 25 (12.0%) Blm<sup>+/−</sup> mice, compared to 10 of 23 (43.5%) WT mice (for 2 WT mice, tissues were not available for histology; 1 Blm<sup>+/−</sup> mouse died of lymphoma).  

**Discussion**  
Loss of BLM helicase activity causes genomic instability and impairs apoptosis, making Bloom syndrome individuals prone to cancer (19, 21, 24, 43, 44). There are conflicting conclusions in the literature as to whether carriers of heterozygous BLM mutations have an increased cancer risk (25, 29, 30, 45–48). Some experiments in mice suggest that BLM dosage is a critical modifier of cancer susceptibility.  

Fig. 3. Blm<sup>−/−</sup> mice exposed to crocidolite asbestos have an altered inflammatory response and shorter survival compared to WT littermates. (A and B) Increase in M1 macrophages in the peritoneal cavity of Blm<sup>−/−</sup> mice (n = 6) compared to WT littermates (n = 9) after treatment with crocidolite (SI Appendix, Fig. S3). (A) Representative flow cytometry dot plot of peritoneal macrophage subpopulations. (B) Percentage of M1 macrophages (CD86<sup>+</sup> CD206<sup>−</sup>); box-and-whisker plots display minimum–maximum range. (C–G) Increased peritoneal cytokine levels in the peritoneal lavage of Blm<sup>−/−</sup> mice compared to WT littermates after crocidolite treatment: (C) TNF-α, (D) IL-1β, (E) IL-3, (F) IL-10, and (G) IL-12(p70) (SI Appendix, Fig. S2); data are shown as mean ± SD; comparisons between heterozygous and WT groups were calculated using a two-tailed unpaired Welch's t test. (H and I) MM incidence (H) and survival (I) in Blm<sup>−/−</sup> and WT mice after exposure to crocidolite. For MM incidence, nonlinear fits were compared using F-test; survival curves were compared using log-rank (Mantel-Cox) test (*P < 0.05; **P < 0.01).
tumorigenesis and constitutional genetics [such as in double-heterozygous breast cancer patients (47)] and that exposure to some viruses might modulate cancer risk in carriers of BLM heterozygous mutations (27).

We discovered that 7 of 155 unrelated mesothelioma patients carried pathogenic heterozygous germline BLM mutations. For 2 of these 7, we had a family pedigree: both patients had relatives who had developed mesothelioma carrying the same BLM mutations as in the proband. The germline c.968A>G mutation found in family 2 was also present in one apparently unrelated mesothelioma patient (Table 1). This significant finding (P = 0.0017), given a 0.00039 mutation probability, together with the high CADD score, supports the pathogenic contributory effect of this mutation to mesothelioma.

In vitro heterozygous BLM mutations induced genomic instability. Mesothelial cells from Blm+/− mice accumulated a higher percentage of micronuclei when exposed to asbestos, and silencing BLM in HM cells exposed to asbestos delayed H2A.X phosphorylation. Generally speaking, diminished DNA repair capacity leads to faster and prolonged induction of γH2A.X. An additional or alternative interpretation to the delayed kinetics of γH2A.X formation (35) induced by crocidolite is that, since single-strand DNA breaks are not potent inducers of γH2A.X foci and replication lesions induced by asbestos may require a second step to produce a lesion that elicits γH2A.X, the second step would likely be DNA replication, which could lead to the formation of DNA DSBs upon replication through a single-strand DNA break. Therefore, delayed H2A.X phosphorylation in cells with reduced BLM levels could also be the consequence of a slower cell cycle and subsequent delayed DNA replication-dependent DSB formation (49).

Moreover, silencing of BLM protected HM cells and macrophages from asbestos-induced apoptosis and protected mesothelioma cells from cell death induced by H2O2 and ceramide. Reduced apoptosis increases the fraction of cells that accumulate genetic damage and that are prone to malignant transformation and helps tumor cells survive chemotherapy (3, 17).

In BLM-silenced macrophages, we found increased release of TNF-α, a cytokine strongly linked to asbesto-mediated carcinogenesis (36, 38–42). Accordingly, Blm+/− mice injected with crocidolite had increased M1 macrophages and higher levels of TNF-α and other proinflammatory cytokines in the peritoneal lavage compared to WT. These results provide a mechanistic rationale for the observation that mice carrying Blm heterozygous mutations are more susceptible to asbestos carcinogenesis compared to WT mice. Indeed, Blm+/− mice exposed to asbestos developed a significantly higher incidence of mesothelioma and had a significantly shorter mesothelioma-specific survival, evidence of GxE. These findings indicate that asbestos exposure increases the risk of mesothelioma in carriers of heterozygous BLM mutations. Since four of nine patients did not report exposure, heterozygous BLM mutations may also increase the risk of mesothelioma per se.

We found that carriers of germline BLM mutations are at increased risk of mesothelioma because: 1) the probability of finding heterozygous pathogenic germline BLM mutations is significantly higher among mesothelioma patients than in the general population (P = 6.7E-10); 2) in affected families, mesothelioma only developed in individuals who inherited the BLM mutation; and 3) the incidence of mesothelioma was higher in Blm+/− mice exposed to asbestos. The observed genomic instability and impairment of asbestos-induced cell death in cells with reduced BLM levels, as well as the altered inflammatory response in human macrophages with reduced BLM levels and in Blm+/− mice, provide a mechanistic rationale for these findings. As for the possible relevance of these findings to other malignancies, it is as yet unknown what the frequency of BLM+/− mutations is in all cancers.

In summary, we identified a mesothelioma family in Turkey with heterozygous germline BLM mutations. This led us to investigate BLM in US mesothelioma families: the combined findings indicated that carriers of heterozygous germline BLM mutations are at increased risk of developing mesothelioma. We validated these findings in a Blm heterozygous mutant mouse model and elucidated mechanisms in tissue culture. We also identified 80 individuals carrying heterozygous BLM loss-of-function variants in a US cohort in Nevada (29,553 individuals) in which, with support from the National Institute of Environmental Health Sciences, we are studying the relationship between germline mutations and exposure to environmental carcinogens. Exposure prevention measures may be warranted for BLM mutant carriers; for example, they may decide not to travel to areas where asbestos and other carcinogenic fibers are abundantly present in the environment (1, 50) and avoid work in trades associated with a risk of exposure to asbestos. They may also benefit from early detection screening along the lines of an NCI clinical trial for BAP1 mutation carriers (ClinicalTrials.gov identifier NCT03830229).

Materials and Methods

Study Oversight and Patients Studied. Collection and use of patient information and samples was in accordance with the Declaration of Helsinki (1995) and the World Medical Association (2013 revision). The full study protocol was approved by our institutional review boards (IRBs): University of Hawaii (IRB no.14406 and 2016–30734), New York University (IRB no. i8896), Hyogo College of Medicine (IRB no. B11H246), and Eskisehir Osmangazi University (IRB no. 80558721-223). All participants (affected and unaffected) provided written informed consent according to the guidelines set forth by our IRBs; HNP recruitment and enrollment is detailed in ref. 18. Details are reported in SI Appendix, Materials and Methods.

Genetic Testing of Germline DNA. Germline DNA was extracted from saliva or peripheral blood (51). For the samples from Turkey, DNA was extracted from peripheral blood, and WGS was conducted by Done Genetik (Istanbul, Turkey). For samples from the United States, peripheral blood DNA extracted from individual patients was used for germline exome sequencing using an Illumina HiSeq.2500 platform. Germline variants found in the mesothelioma cohort (n = 122) were gathered from variant call format and quality-control files and consolidated into a text file. The variants were required to be high-confidence, i.e., meeting the following criteria: a minimum of 10× total coverage, a minimum of 3× variant coverage, a minimum of 0.25 variant allele frequency, not called by Platypus (52) alone, and present in at most 20% of all samples. WGS was performed according to manufacturer protocol on an Illumina HiSeq.2500 using paired-end 150-bp runs. Details are reported in SI Appendix, Materials and Methods.

In Vivo Experiments in Blm−/− Mice Exposed to Asbestos. Blm−/− mice were generated by and obtained from author J.G. (26). Details are reported in SI Appendix, Materials and Methods.

Statistical Analyses. To calculate the probability of n or more mutations in the given sample, all statistical tests were based on a binomial distribution with parameters N and p, where N is the total number of patients and p is the probability of having the mutation. The binomial distribution gives the discrete probability distribution of obtaining exactly n events out of N possible, where the probability of an event is p. In the long-term exposure to asbestos fibers experiment, MM incidence nonlinear-fit and survival curves were compared by t-test and log-rank (Mantel–Cox) test, respectively. In vitro data are presented as mean ± SD unless otherwise specified. P values were calculated using two-tailed unpaired Welch’s t test unless otherwise specified. P values <0.05 were considered statistically significant and marked with asterisks as indicated in the figure legends.

Additional Materials and Methods are described in SI Appendix, Materials and Methods.

Data Availability. All study data are included in the article and SI Appendix.

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