Inherited Predisposition to Malignant Mesothelioma and Overall Survival Following Platinum Chemotherapy: Supplementary Material

METHODS

Genomics. Human subjects committees of the National Cancer Institute (NCI), the University of Chicago (UC), and the University of Washington (UW) approved the studies; all patients provided written informed consent. For patients from the NCI, genomic DNA isolated from peripheral blood was sequenced using the multi-gene panel BROCA v10, which includes 73 genes involved in DNA repair and/or in inherited predisposition to solid tumors (Table S8)[1]. BROCA detects all classes of mutations: point mutations, small insertions and deletions, and large genomic duplications or deletions. Sequencing, bioinformatics, and variant interpretation were carried out as previously described[2]. For patients from UC, genetic testing was carried out as previously described[3]. For all patients, analyses of germline mutations included only unambiguously damaging mutations: frameshifts, nonsense mutations, complete gene deletions, internal genomic deletions leading to truncations, splice mutations shown experimentally to lead to loss of functional transcripts, and missense mutations shown experimentally to damage protein function. That is, patients with variants of unknown significance (VUS), but with no clearly damaging mutation in any gene, were defined as having no detectable germline mutation. No patients carried missense mutations of uncertain significance in *BAP1*. Frequencies of germline mutations in the mesothelioma patients were compared to frequencies in two series of controls: 27173 individuals of European ancestry in the Exome Aggregation Consortium (ExAC) database, after removing exomes contributed by The Cancer Genome Atlas (TCGA), and 7325 participants of European ancestry in the Women's Health Initiative (WHI) who were older than age 70y and never developed cancer[4,5,6]. For patients with inherited *BAP1* mutations, whole exome sequencing was carried out on DNA extracted from formalin fixed paraffin embedded (FFPE) tumor tissue, as previously described[7].

Statistics. Survival was evaluated by Kaplan-Meier methods with survival curves compared by 2-tailed log-rank tests, with exact tests if there were <10 events in any group. Survival data was censored as of June 30, 2017 for NCI patients and as of September 30, 2018 for UC patients. Associations between allele frequencies and clinical and demographic characteristics were compared by two-tailed chi-square tests or Fisher's exact tests, as appropriate. Allele frequencies were compared gene by gene, adjusted for differences in ancestry between case and control series. Effect of genotype on survival after adjusting for age at diagnosis and gender was assessed by multiple logistic regression analysis, comparing patients who either died by 2, 3, or 5 years or who survived longer than these time points.

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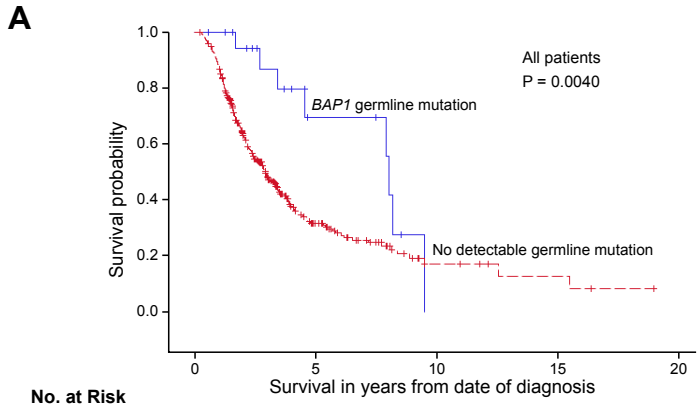
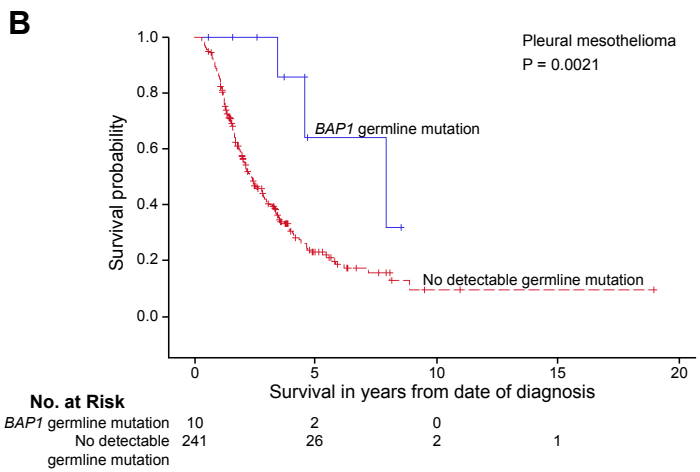

A
All patients
P = 0.0040
BAP1 germline mutation
No detectable germline mutation
Survival probability
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
Survival in years from date of diagnosis
No. at Risk
BAP1 germline mutation 21 6 0
No detectable germline mutation 345 51 7 3


B
Pleural mesothelioma
P = 0.0021
BAP1 germline mutation
No detectable germline mutation
Survival probability
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
Survival in years from date of diagnosis
No. at Risk
BAP1 germline mutation 10 2 0
No detectable germline mutation 241 26 2 1

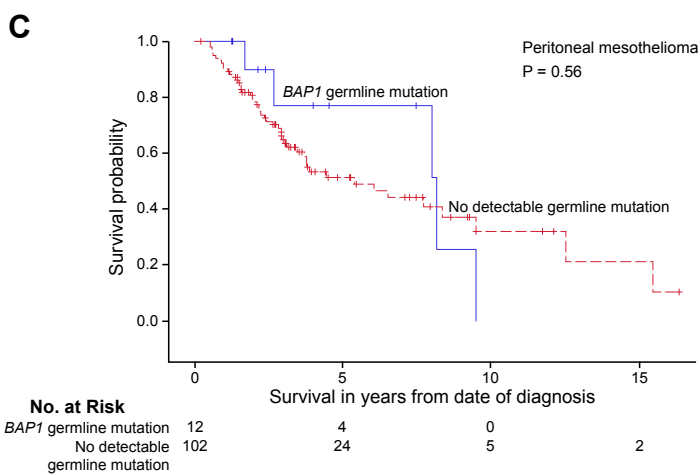

C
Peritoneal mesothelioma
P = 0.56
BAP1 germline mutation
No detectable germline mutation
Survival probability
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
Survival in years from date of diagnosis
No. at Risk
BAP1 germline mutation 12 4 0
No detectable germline mutation 102 24 5 2

Figure S1. Survival of patients with mesothelioma treated with platinum chemotherapy, by patient's *BAP1* genotype and primary site of tumor. Survival of patients with an inherited damaging mutation in *BAP1* is indicated in blue; survival of patients with no inherited mutation in any targeted gene is indicated in red. (**A)** All mesothelioma patients with versus without inherited mutation. Median survival: 8.0 vs 2.9 years, P=0.004. (**B)** Pleural mesothelioma patients with versus without inherited mutation. Median survival: 7.9 vs 2.4 years, P=0.002 (**C)** Peritoneal mesothelioma patients with versus without inherited mutation. Median survival: 8.2 vs 5.4 years, P=0.56.

Table S1. Personal and family history of cancers for mesothelioma patients with germline mutations in targeted genes

ID	Sex	Age dx	Asbestos	Site	Gene	Mutation	Protein	Personal history other cancers	Family history mesothelioma	Family history other cancers
MNH254	M	48	No	Peritoneum	*BAP1*	c.122+1G>A (exon3)		Ocular melanoma, cutaneous melanoma, basal cell, lung, meningioma	-	Gastric, lung
MNH207	M	69	Yes	Peritoneum	*BAP1*	c.155G>A (exon4)	W52X	Prostate	-	Breast, melanoma
MNH182	F	59	Yes	Pleura	*BAP1*	c.436insA (exon6)	R146fs	Breast	-	Breast, ovary, lung, pancreas, testis
MNH104	M	56	Unknown	Peritoneum	*BAP1*	c.437+1G>C (exon6)		Bladder, skin	-	None
MNH007*	F	50	No	Pleura	*BAP1*	c.659+1G>C (exon8)		-	Father, 2 sisters	Breast, basal cell, kidney, ocular melanoma
MNH029*	F	50	Unknown	Pleura	*BAP1*	c.659+1G>C (exon8)		-	Father, 2 sisters	Breast, basal cell, kidney, ocular melanoma
MNH248	F	50	No	Pleura	*BAP1*	c.659+1G>T (exon8)		-	-	Melanoma
MNH107	F	46	Yes	Pleura	*BAP1*	c.836C>A (exon10)	S279X	-	-	Prostate
MNH018	M	44	Unknown	Peritoneum	*BAP1*	c.1717delC (exon13)	L573fs	-	-	Gastric
MNH088*	F	55	Unknown	Pleura	*BAP1*	c.1717delC (exon13)	L573fs	Bladder, melanoma, breast	Mother, maternal cousin	Melanoma, ocular melanoma, skin
MNH177	M	56	Yes	Peritoneum	*BAP1*	c.1717delC (exon13)	L573fs	-	-	Breast, thyroid
MNH227*	M	51	Unknown	Peritoneum	*BAP1*	c.1717delC (exon13)	L573fs	Bladder	Paternal aunt and cousin	Hodgkin lymphoma, ocular melanoma, nasopharynx
MNH240	F	60	Yes	Pleura	*BAP1*	c.178C>T (exon4)	R60X	Cutaneous melanoma	-	Breast, colon, kidney, melanoma
MNH116	F	53	Yes	Pleura	*BAP1*	c.1882delTCAC (exon14)	S628fs	Basal cell	Father	Ocular melanoma, skin
MNH159	F	59	Unknown	Pleura	*BAP1*	c.1938T>A (exon15)	Y646X	-	Father, brother, sister	Ocular melanoma
MNH074	M	48	Yes	Peritoneum	*BAP1*	c.1956delGG (exon15)	E652fs	Thyroid	Mother, brother	-
MNH199	M	55	Yes	Peritoneum	*BAP1*	c.2050C>T (exon16)	Q684X	-	-	Breast, brain, lung
MNH195	F	47	Yes	Peritoneum	*BAP1*	c.2188T>A (exon17)	X730R	Squamous cell	-	Colon
MNH086	M	83	Yes	TV	*BRCA2*	c.5946delT (exon11)	S1982fs	Basal cell, colon	-	Breast, lung
MNH216	F	63	Unknown	Peritoneum	*CHEK2*	c.538C>T (exon4)	R180X	-	-	Lung, melanoma, lymphoma
MNH011	F	50	Yes	Peritoneum	*CHEK2*	c.1036C>T	R346C	-	-	Lymphoma, bladder, breast
MNH065	M	67	Yes	Pleura	*CHEK2*	c.1229delC (exon12)	T410fs	Basal cell	-	Breast, colon
MNH020	M	66	Yes	Pleura	*CHEK2*	del exons 9-10 (5395 bp)		-	-	Melanoma
MNH052	M	79	Yes	Pleura	*CHEK2*	del exons 9-10 (5395 bp)		Prostate		Pancreas
MNH145	M	59	Yes	Peritoneum	*MLH1*	del exon 16 - 3'UTR	del aa 548-756	-	-	Colon, ovary, gastric, breast, melanoma, liver
MNH242	F	60	No	Pleura	*MRE11A*	c.504del8 (exon6)	L168fs	Melanoma	-	Breast, colon, lung, skin
MNH021	F	60	Yes	Peritoneum	*PALB2*	c.2092delC (exon5)	L698fs	-	-	Bladder, breast, gastric, pancreas, prostate
MNH092	F	23	Unknown	Peritoneum	*PALB2*	c.3048delT (exon10)	F1016fs			
MNH153	M	54	Yes	Peritoneum	*POT1*	c.669C>G (exon9)	Y223X	Thyroid	-	Site unknown
MNH041	F	27	No	Pleura	*TP53*	c.742C>T (exon7)	R248W	-	-	None: *de novo* mutation

*MNH007 and MNH029 are sisters; MNH088 and MNH227 are cousins

Table S2. BAP1 mutations in tumors

				BAP1 germline mutation				BAP1 point mutations in tumors				BAP1 somatic gains and losses in tumors		
Patient ID	tumor content	mean coverage targeted sites	% of positions ≥20x	Position (hg19)	cDNA	Protein	total|var reads in tumor	Position (hg19)	cDNA	Protein	total|var reads	Position (hg19)	Type	median log2 ratio
MNH007	0.30	166	95.7	chr3:52440844	c.659(+1)G>C		133|71	chr3:52439196	c.1046delA	N349fs	131|35			
MNH018	0.80	142	95.6	chr3:52437444	c.1717delC	L573fs	213|190					unbalanced duplication		
MNH074	1.00	144	95.3	chr3:52436821	c.1956delGG	E652fs	105|22					chr3:52,388,360-52,437,492	loss	-0.31
MNH088	0.72	59	81.3	chr3:52437444	c.1717delC	L573fs	77|49	chr3:52441217	c.553G>A	G185R	21|7	chr3:50,138,355-57,472,326	imbalance	0.08
MNH104	0.29	68	74.8	chr3:52441414	c.437(+1)G>C		177|49	chr3:52439877	c.834_835delTG	E278fs	67|22	chr3:51,895,441-52,934,690	gain	0.41
MNH107	0.71	63	92.3	chr3:52439876	c.836C>A	S279X	130|57	chr3:52442491	c.254A>T	Q85L	128|7			
MNH177	0.60	111	94.3	chr3:52437444	c.1717delC	L573fs	114|91					chr3:52,326,997-52,440,657	loss	-0.52
MNH182	0.70	151	95.4	chr3:52441415	c.436dupA	R146fs	195|100	chr3:52443866	c.20_29del	E7fs	82|18	chr3:52,387,483-52,439,619	loss	-0.35
MNH207	0.55	140	95.4	chr3:52442590	c.155G>A	W52X	321|240					chr3:52,386,421-52,440,160 chr3:52,440,160-52,445,415	loss gain	-0.46 0.35
MNH227	0.85	117	94.1	chr3:52437444	c.1717delC	L573fs	157|71	chr3:52437549	c.1612delC	L538fs	168|23	chr3:52,440,657-52,445,415	gain	0.58
MNH240	0.25	104	93.9	chr3:52442567	c.178C>T	R60X	212|103	chr3:52442492	c.253C>T	Q85X	145|41	chr3:52,326,997-52,440,657	loss	-0.66
MNH254	0.95	130	96.1	chr3:52443569	c.122(+1)G>A		145|88					chr3:52,393,077-52,440,160	loss	-0.32

Table S3. Familial mesothelioma among patients with no detected inherited mutations

Patient	Site of disease	Histology	Age dx (years)	Sex	Asbestos exposure of proband	Ever smoked	Relative with mesothelioma	Asbestos exposure of relative
MNH056	Pleural	Epitheloid	51	F	None known	No	Uncle	Pipe insulator >30 years
MNH059	Pleural	Epitheloid	53	M	Self-employed plumbing and heating, 15 years' exposure	Yes	Uncle	Worked entire adult life in garage
MNH125	Pleural	Sarcoma-toid	61	M	Retired asbestos insulator, 36 years' exposure	No	Father	Worked entire adult life as asbestos insulator, same company as son
MNH139	Pleural	Epitheloid	71	M	Career Navy, 8 years' exposure	Yes	Uncle	Not sure if uncle's cancer was mesothelioma or lung, no information on exposure
MNH144	Peritoneal	Epitheloid	12	M	None known	Yes	Grandfather	Long term exposure at power plant documented by asbestos screening program, 20y history of pleural plaques, still alive
MNH245	Pleural	Epitheloid	42	F	None known	No	Mother	Died of pleural mesothelioma, no information on exposure

Table S4. Numbers of unrelated cases and controls with inherited loss-of-function mutations in genes associated with malignant mesothelioma

	Cases				Controls*		OR (95% CI)	P
	NCI	UC	Total	%	Source	N		
Total**	239	198	432		ExAC	27173		
					WHI	7325		
BAP1	16	6	22	5.1	ExAC	1	1458 (196, 10843)	< 10E-30
					WHI	1	393 (53, 2923)	< 10E-30
BRCA2	1	3	4	0.9	ExAC	90	2.80 (1.02, 7.66)	0.036
					WHI	12	5.67 (1.82, 17.65)	0.0007
CHEK2	5	3	8	1.9	ExAC	154	3.29 (1.61, 6.75)	0.00004
					WHI	32	4.28 (1.96, 9.34)	0.0006
MLH1	1	0	1	0.2	ExAC	3	20.9 (2.16, 201.0)	0.00008
					WHI	-		
MRE11A	1	1	2	0.5	ExAC	18	6.98 (1.62, 30.19)	0.002
					WHI	5	6.78 (1.31, 35.04)	0.008
PALB2	2	0	2	0.5	ExAC	24	5.24 (1.24, 22.23)	0.012
					WHI	7	4.84 (1.00, 23.37)	0.030
POT1	1	0	1	0.2	ExAC	7	8.94 (1.11, 72.84)	0.013
					WHI	-		

*Control series: Exome Aggregation Consortium (ExAC) with exomes from TCGA removed, N = 27,173; Women Health Initiative (WHI) participants, age >70y and cancer free, N = 7,325. All patients with damaging mutations were of European ancestry, so only European ancestry controls were included in analyses.

**Total excludes duplicate records for 5 patients seen at both NCI and UC, and excludes second relatives from each of two families

Table S5. Germline mutations and survival following platinum-based chemotherapy

	All patients	Pleural disease	Peritoneal disease
Number of patients			
Germline mutation in *BAP1**	21	10	12
Germline mutation, any gene*	40	20	21
No detectable germline mutation	345	241	102
Median years survival [95%CI]			
Germline mutation in *BAP1*	8.0 [3.4, 9.5]	7.9 [3.4, nc]	8.2 [1.7, 9.5]
Germline mutation, any gene	8.0 [4.6,14.8]	7.9 [3.4, nc]	8.2 [3.4, 14.8]
No detectable germline mutation	2.9 [2.4, 3.5]	2.4 [2.0, 2.9]	5.4 [3.5, 9.5]
Proportion surviving 5 years [95%CI]			
Germline mutation in *BAP1*	0.70 [0.37, 0.88]	0.62 [0.15, 0.90]	0.77 [0.35, 0.94]
Germline mutation, any gene	0.62 [0.40, 0.77]	0.62 [0.28, 0.84]	0.63 [0.33, 0.82]
No detectable germline mutation	0.32 [0.26, 0.38]	0.23 [0.17, 0.30]	0.52 [0.39, 0.62]

*one patient with a *BAP1* mutation had both pleural and peritoneal disease
nc, not calculable

Table S6. Survival following platinum chemotherapy of patients with germline mutations.

Patient ID	Mesothelioma origin	Germline mutation	Status	Survival in days*
MNH195	peritoneal	BAP1	A	3467
MNH107	pleural	BAP1	A	3124
MNH227	peritoneal	BAP1	A	2731
MNH248	pleural	BAP1	A	1714
UC049	pleural+peritoneal	BAP1	A	1667
MNH207	peritoneal	BAP1	A	1464
MNH159	pleural	BAP1	A	1351
MNH088	pleural	BAP1	A	943
MNH254	peritoneal	BAP1	A	874
UC041	peritoneal	BAP1	A	788
MNH182	pleural	BAP1	A	575
UC238	peritoneal	BAP1	A	470
UC221	peritoneal	BAP1	A	467
MNH240	pleural	BAP1	A	219
MNH177	peritoneal	BAP1	D	3488
MNH018	peritoneal	BAP1	D	2992
MNH104	peritoneal	BAP1	D	2952
MNH116	pleural	BAP1	D	2909
MNH029	pleural	BAP1	D	1672
MNH007	pleural	BAP1	D	1271
MNH074	peritoneal	BAP1	D	984
UC102	peritoneal	BAP1	D	626
MNH199	peritoneal	BAP1	D	152
UC170	peritoneal	ATM	A	2147
UC258	pleural	ATM	D	496
MNH086	TV	BRCA2	A	977
UC191	pleural	BRCA2	A	561
UC241	peritoneal	BRCA2	D	155
UC061	peritoneal	CDKN2A	A	1571
UC265	pleural	CDKN2A	A	1437
MNH011	peritoneal	CHEK2	A	4640
MNH052	pleural	CHEK2	A	1180
MNH216	peritoneal	CHEK2	D	1412
MNH065	pleural	CHEK2	D	486
MNH020	pleural	CHEK2	D	266
MNH145	peritoneal	MLH1	D	946
MNH242	pleural	MRE11A	A	244
UC081	pleural	MSH6	A	2539
MNH092	peritoneal	PALB2	D	5410
MNH021	peritoneal	PALB2	D	1687
MNH153	peritoneal	POT1	A	1149
MNH041	pleural	TP53	A	4847
UC240	pleural	VHL	A	378
UC059	peritoneal	WT1	D	1435

*Days between treatment and last follow up, for patients still alive (A), or between treatment and death, for deceased patients (D).

Table S7. Independent effects of age at diagnosis, gender, and genotype on death within 3 years of diagnosis for patients with pleural mesothelioma treated with platinum-based chemotherapy.*

Parameter	Estimate	S.E.	P	OR [95% CI]
Intercept	-2.16	0.73	0.003	
Diagnosis ≥ 60 y	0.97	0.33	0.003	2.64 [1.39, 5.01]
Gender male	0.69	0.33	0.038	2.00 [1.04, 3.86]
No detectable inherited mutation	1.52	0.70	0.031	4.56 [1.15, 18.06]

*Includes 241 patients with death within 3 years or ≥3 years follow-up; 40 patients alive <3 years at last follow-up have undetermined 3-year survival status.

Table S8. Genes sequenced using BROCA v10

AKT1	FLCN	POT1
APC	GALT12	PPMID
ATM	GEN1	PRKAR1A
ATR	GREM1	PRSS1
AXIN2	HOXB13	PTCH1
BAP1	KIT	PTEN
BARD1	MEN1	RAD51B
BLM	MET	RAD51C
BMPR1A	MITF	RAD51D
BRCA1	MLH1	RB1
BRCA2	MRE11A	RECQL
BRCC3	MSH2 (+EPCAM)	RET
BRIP1	MSH6	RINT1
CDH1	MUTYH	RPS20
CDK4	NBN	SDHA
CDKN2A	NF1	SDHB
CHEK1	NTHL1	SDHC
CHEK2	PALB2	SDHD
CTNNA1	PALLD	SLX4
ENG	PDGFRA	SMAD4
FAM175A (ABRAXAS)	PIK3CA	SMARCA4
FANCA	PMS2	STK11
FANCM	POLD1	TP53
FH	POLE	VHL
		XRCC2