# Update on Genetic Evidence in Tort Cases

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# ToxicoGenomica

**Multidisciplinary group of geneticists, scientific consultants, and counsel offering consulting and expert services in genomics & systems biology regarding toxins**

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# Next ToxicoGenomica Webinar – Thursday Nov. 20

- The next ToxicoGenomica webinar with Perrin Conferences is set for Thursday, Nov. 20
  - 2-3:40 eastern
- Title: "Looking Ahead to 2026: Accelerating Genomics in Personal Injury Cases"
- Free registration is at the link:
  - https://www.perrinconferences.com/webinars/112025/

# Examples of Uses of Genetic Evidence In Trials, Dispositive Motions and MDLs

# Genetic Evidence is in Fact Admitted into Evidence, Contrary to Arguments by Some

- Some lawyers for some plaintiffs have asserted that courts do not admit genetic evidence
  - that assertion is false

- Genetic and genomic evidence and argument has been admitted in numerous asbestos and benzene cases and trials, as well as birth defect cases, medical malpractice cases, chemical exposure cases and MDLs involving various drugs

- Plaintiff's counsel sometimes have offered genetic evidence to try to support claims
  - for example, Kazan firm presented genetic evidence in Ortwein trial; see this summary article
  - for example, Mark Lanier offered genetic evidence in the Ingham trial ($4+ billion verdict)

# Courts Have Granted Summary Judgment Based on Genetic Evidence

- State and federal courts have granted summary judgment motions based on genetic evidence
- The earliest example is the the seminal 2006 case in which the defense used genetic testing to establish that terrible malformations of children were caused by a genetic mutation that causes CHARGE syndrome rather than exposure to a fungicide*, Bowen v. E.I. DuPont de Nemours & Co.*, 906 A.2d 787 (2006).
- A recent example is a medical malpractice case, *Ortega v. United States*, 2021 U.S. Dist. LEXIS 188969, 2021 WL 4477896. There, the Court stated:
  - "Because all evidence in the record indicates that J.A.O.'s neuromuscular failure was caused by a congenital [genetic] condition rather than by negligence on the part of the healthcare providers, both motions [for summary judgment] are granted."

# MLDs Involving Genetic Evidence

| | |
|---|---|
| In re Zostavax (Zoster Vaccine Live) Prods. Liab. Litig., 2024 U.S. App. LEXIS 17369, 2024 WL 3423709 | Dismissal of over 1,700 claims when plaintiffs failed to present a specific form of genetic testing results to establish whether a virus was caused by ordinary chicken pox or a vaccine |
| In re Acetaminophen — ASD-ADHD Prods. Liab. Litig., 2024 U.S. Dist. LEXIS 121259, 2024 WL 3357608 | Plaintiff's expert barred under Rule 702 due to failure to meaningfully address genetic factors as a cause of ADHD rather than use of acetaminophen; extended discussion of genetic variables |
| In re Cpap, 2023 U.S. Dist. LEXIS 193561 | Evaluating adequacy of allegations of genetic harm |

# MDLs that Inevitably Will Involve Genomic Evidence

| | |
|---|---|
| IN RE: DEPO-PROVERA (DEPOT MEDROXYPROGESTERONE ACETATE) PRODUCTS LIABILITY LITIGATION | MDL involves claims that use of certain amounts of Depo-Provera cause brain tumors known as meningiomas. Plaintiff oriented web site page acknowledges genetic causes of meningiomas. "Risk factors for meningioma stem from both genetic and environmental origins. The primary genetic risk is neurofibromatosis type 2 (NF2), significantly raising the chances of developing multiple meningiomas. Other genetic conditions associated with an increased risk include Gorlin syndrome, multiple endocrine neoplasia type 1 (MEN1), Turcot syndrome, Lynch syndrome, Li-Fraumeni syndrome, Cowden syndrome, and Von Hippel-Lindau disease." https://www.lawsuit-information-center.com/depo-provera-lawsuit.html |
| In re: Hair Relaxer Marketing Sales Practices and Products Liability Litigation, case number 1:23-cv-00818 (N.D. IL) | Claims that breast and ovarian cancers (among others) are caused by hair relaxers marketed mainly to women of color, but those cancers also are caused by pathogenic mutations in genes known as *BRCA1* and *2*, among others. |

# “Genetic Defenses” are Not All the Same

# Two Main Types of "Genetic Defenses"

- Some stakeholders incorrectly argue as if all "genetic causation defenses" are the same

- Generally speaking, "genetic causation defenses" fall into two different categories, with very different amounts of objective, reproducible supporting evidence:

  - inherited (germline) mutations
    - sequencing of germline tissue (usually blood) and other actions can provide abundant objective, reproducible evidence of an inherited mutation and pathogenicity

  - somatic mutations that are present in a tumor and occurred during life
    - sequencing of tumor typically does not show when or why mutation occurred
    - more specifics about the when and how of somatic mutations may arise in 5-10 years with improvements in tools and technologies

# Defense Based on Inherited (Germline) Mutations

- A genetic defense best based on the results of germline genetic sequencing results specific to the plaintiff
  - key aspect of this defense is based on analysis of data/results of germline sequencing
    - well accepted germline inferences can be drawn from tumor sequencing and variant allelic frequency (VAF) %s between 30-70%
- To the best of my knowledge, no one has tried a germline genetic defense to verdict
- It is certain that no one has tried to verdict a case in which Dr. Len van Zyl has been called to testify

# Defense Based on Somatic Mutations – "Replication Error"

- In contrast, the other "bucket" of genetic defenses focuses on somatic mutations that occur during life

- A "replication error" defense typically is based mainly on the general principles that:
  - pathogenic somatic mutations can and do occur when cells divide themselves (mitosis) in order to produce new cells (replication), and
  - some replication error mutations that were not repaired may cause a cancer
  - to oversimplify, some key aspects of this defense are based on the so-called "bad luck" studies by Drs. Vogelstein, Tomasetti and others

- To the best of my knowledge, all "genetic causation defense" mesothelioma trials that have gone to verdict have been "replication error" defenses, sometimes with some additional facts unique to the plaintiff

# Much More Objective, Reproducible Evidence in Plaintiff Specific Germline Mutation Cases

- Objective reproducible plaintiff specific evidence can be presented regarding germline mutations:
  - personal and familial cancer histories, including age of onset and other data pertinent to the cancer
  - personal and familial medical histories showing the presence of non-cancer conditions and diseases caused by mutations in genes that also cause cancers
  - results of germline sequencing that indisputably establish the presence of one or more pathogenic germline mutations in one or more genes

# More Subsets of Objective Reproducible Evidence in Plaintiff Specific Germline Genetic Defenses

- More subsets of objective, reproducible evidence with a pathogenic germline mutation defense:
  - results of reproducible, software driven CADD score analyses which provide a metric that ranks the pathogenicity of mutations in "cancer genes"
  - ExAC scores showing relative rarity of mutation
  - results of recent in silico and in vitro studies that use saturation gene editing tools to assess whether a particular mutation in a particular gene is in fact pathogenic (e.g. Waters 2024 study of about 99% of the possible mutations of *BAP1*)
  - results of somatic sequencing (tumor sequencing) with VAF%s (usually 30-70%) that establish the likely presence of one or more germline mutations in "cancer genes"

# Data Can Be Key

# Data Can Be Key

- Experts and lawyers often need to focus on data published as part of studies rather than looking only for sound bites
- Researchers often do not include statements explaining conclusions that are obvious from data they publish in article
- Data in online "supplemental materials" may be key

# Data Not Gathered in the Past

- Plaintiff lawyers can point out that past medical histories did not include questions about talc use
- Defense lawyers can point out that past medical treatment did not seeking information on pathogenic germline mutations
- And, even after genetic data was obtained, it was often highly limited (e.g. exome panel sequencing) rather than whole genome sequencing
- In the past, and even today, many doctors and researchers did not use CADD scores or other in silico tools to evaluate the pathogenicity of the germline mutations in mesothelioma families
  - sometimes used “asbestos must have done it” assumptions parallel to Helsinki 1997

# Some Key Data for Defense

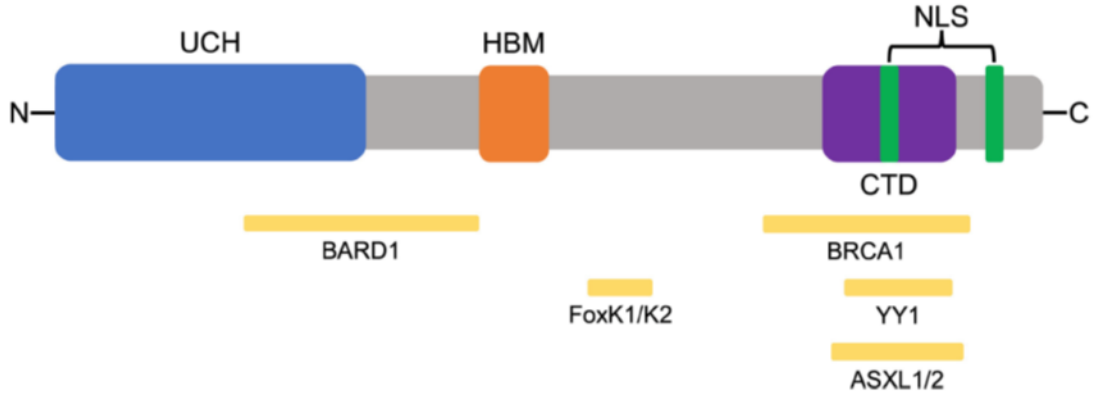
- Zero peer reviewed articles state that genetic abnormalities cannot cause mesotheliomas

- Helsinki 1997 conference:
  - 1) preceded completion of Human Genome Project,
  - 2) included zero geneticists,
  - 3) preceded 2011 publication of article by Drs. Testa and Carbone regarding association between BAP1 mutations and mesothelioma,
  - 4) preceded other 2011 articles showing association between other cancers and BAP1 mutations, and
  - 5) preceded widespread WGS, which has produced key data and findings

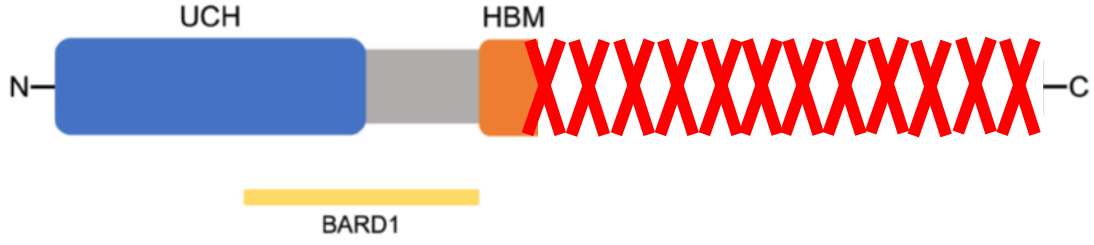
# “Mutations” are Not All the Same; Get the Data

# Each Gene Can Have Many Different Mutations; They Are Not Fungible

Wild Type (Normal) BAP1 Protein Structure (729 amino acids)




The germline *BAP1* p.Arg385Ter null mutation truncates (terminates) the BAP1 protein at amino acid 385, resulting in a BAP1 (1-385) mutant protein that lacks more than half (53%) of its coding region. The BAP1 protein encoded by this mutant gene is incapable of carrying out normal *BAP1* functions.



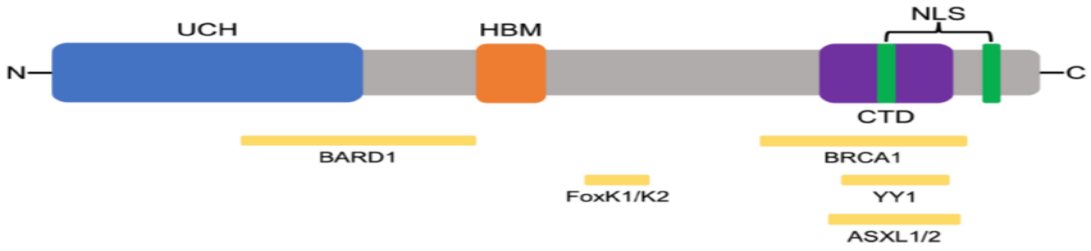

https://www.ncbi.nlm.nih.gov/clinvar/variation/485271/

# Has/Can Opposing Study or Expert Evaluated Mutation?

Location of mutation impacts impairment/function

Ask: was that done in a prior meso study? Can opposing expert do it?

Wild Type (Normal) BAP1 Protein Structure (729 amino acids)

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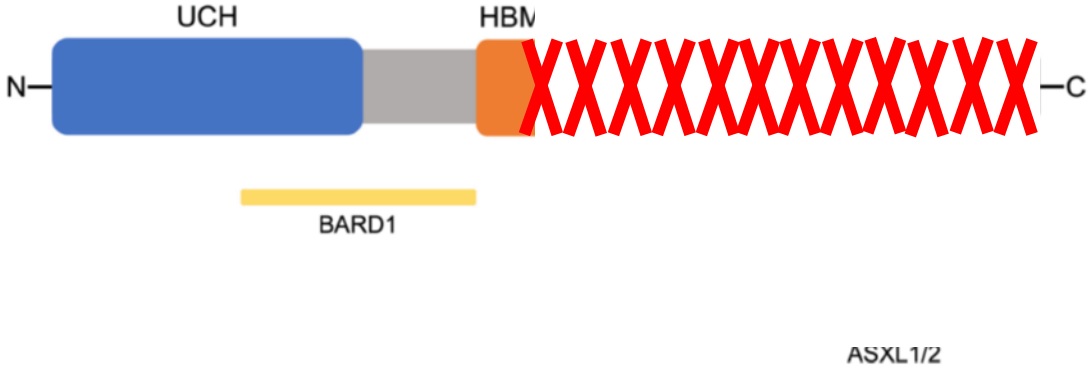


https://www.ncbi.nlm.nih.gov/clinvar/variation/485271/

# Genetics and California Evidence Code Section 801.1

- California plaintiff's bar obtained Section 801.1 after Zimmer ruling in appellate court

- Consider possible application to genetics using subpart (b)
  - **801.1. Expert opinions regarding medical causation.**
  - (a) In a general civil case, as defined in Rule 1.6 of the California Rules of Court, where the party bearing the burden of proof proffers expert testimony regarding medical causation and where that party's expert is required as a condition of testifying to opine that causation exists to a reasonable medical probability, the party *not* bearing the burden of proof may offer a contrary expert only if its expert is able to opine that the proffered alternative cause or causes each exists to a reasonable medical probability, except as provided in subdivision (b).
  - (b) Subdivision (a) does not preclude a witness testifying as an expert from testifying that a matter cannot meet a reasonable degree of probability in the applicable field, and providing the basis for that opinion.

- For example, plaintiff experts tend to treat mutations as fungible, but they are not

# Objective, Reproducible Metrics and California Evidence Code Section 801.1

  - (b) Subdivision (a) does not preclude a witness testifying as an expert from testifying that a matter cannot meet a reasonable degree of probability in the applicable field, and providing the basis for that opinion.

- Defense lawyers should keep in mind that plaintiff experts tend to treat mutations as fungible but they are not

- Experts called by defense can testify to existence of objective, reproducible metrics such as:
  - CADD scores regarding pathogenicity of mutation
  - ExAC scores  regarding rarity of mutation

- Defense can move to bar or cross-examine due to plaintiff expert failure to introduce objective mutation specific evidence for mutations for *BAP1* and other genes,

# CADD Scores are in Published Studies

# Wu 2025 (NCI) – CADD Scores for Gene Variants



**Table 1.** Summary of Subjects and Corresponding BAP1 Mutations and Their Significance

| ID | Sex | Age at Enrollment | Smoking History | Germline Mutation | Consequence | Functional Score (NG) | CADD Score | CLIN_SIG | ClinVar_CLNSIG_slim | Clinical Group | Cohort |
|---|---|---|---|---|---|---|---|---|---|---|---|
| S1 | F | 65 | N | c.436dupA | depleted | −0.189332075 | 16.12 | Unobserved | Pathogenic (P) | M2 | NCT04431024 |
| S2[a] | M | 66.2 | N | c.855dupC | Frameshift | −0.1083403 | N/A | Pathogenic | Likely Pathogenic (LP) | M2 | NCT04431024 |
| S3 | M | 51.9 | N | c.830_831del | Stop gained | −0.1109776 | N/A | Pathogenic | P/LP | M2 | NCT04431024 |
| S4 | M | 68.5 | N | c.1938T>A | Stop gained | −0.1315452 | 36 | Pathogenic | P/LP | M1 | NCT04431024 |
| S5 | M | 46.3 | Y | c.1938T>A | Stop gained | −0.1315452 | 36 | Pathogenic | P/LP | M1 | NCT04431024 |
| S6 | M | 47.7 | N | c.1938T>A | Stop gained | −0.1315452 | 36 | Pathogenic | P/LP | M1 | NCT04431024 |
| S7 | M | 69.5 | N | c.1153C>T | Stop gained | −0.1109776 | 36 | Pathogenic | P/LP | M2 | NCT04431024 |
| S8 | M | 44.8 | Y | c.1938T>A | Stop gained | −0.1315452 | 36 | Pathogenic | P/LP | M1 | NCT04431024 |
| S9 | F | 42.7 | Y | c.778C>T | Stop gained | −0.02900829 | 39 | Pathogenic | P/LP | M1 | NCT04431024 |
| S10 | M | 64 | N | c.1717delC | Frameshift | −0.038271807 | 32 | Pathogenic | P/LP | M2 | NCT04431024 |
| S11 | M | 65.7 | Y | c.1717delC | Frameshift | −0.038271807 | 32 | Pathogenic | P/LP | M2 | NCT04431024 |
| S12 | M | 68.5 | N | c.2056+1G>C | Splice donor | −0.1364949 | N/A | Likely pathogenic | P/LP | M2 | NCT04431024 |
| S13 | F | 33.2 | N | c.1777C>T | Stop gained | −0.0794287 | 38 | Pathogenic | P/LP | M1 | NCT04431024 |
| S14 | F | 63.4 | N | c.458delC | Frameshift | −0.1974506 | N/A | Pathogenic | P/LP | M2 | NCT04431024 |
| S15 | F | 36 | N | c.1153C>T | Stop gained | −0.1109776 | 36 | Pathogenic | P/LP | M2 | NCT04431024 |
| S16 | M | 37.3 | Y | c.178C>T | Stop gained | −0.070179237 | 36 | Pathogenic | P/LP | M2 | NCT04431024 |
| S17 | M | 53 | N | c.2050C>T | Stop gained | −0.1132796 | 42 | Pathogenic | P/LP | M1 | NCT04431024 |
| S18[a] | M | 49.8 | N | c.119_120del | Frameshift | −0.2098157 | N/A | Pathogenic | P/LP | M2 | NCT04431024 |
| S19 | F | 52.2 | Y | c.2050C>T | Stop gained | −0.1132796 | 42 | Pathogenic | P/LP | M2 | NCT04431024 |
| S20 | F | 47.4 | N | c.2050C>T | Stop gained | −0.1132796 | 42 | Pathogenic | P/LP | M1 | NCT04431024 |
| S21 | M | 39 | Y | c.292A>C | Missense | −0.15098855 | 26.8 | N/A | Uncertain | M2 | NCT04431024 |
| S22 | F | 50.6 | N | c.1203dup | Frameshift | −0.2320384 | 26.7 | Pathogenic | P/LP | M1 | NCT04431024 |
| S23 | M | 34.3 | N | Exon 1-14 deletion | N/A | −0.177246202 | N/A | Pathogenic | P/LP | M2 | NCT04431024 |
| S24 | F | 59.5 | N | c.1203dup | Frameshift | −0.2320384 | 26.7 | Pathogenic | P/LP | M1 | NCT04431024 |
| S25[a] | F | 30.8 | Y | c.1203dup | Frameshift | −0.2320384 | 26.7 | Pathogenic | P/LP | N/A | NCT04431024 |
| S26 | F | 73.3 | N | c.1153C>T | Stop gained | −0.1109776 | 36 | Pathogenic | P/LP | M2 | NCT04431024 |
| S27 | F | 35.2 | N | Partial deletion (exons 10-11) | N/A | N/A | N/A | N/A | N/A | M0 | NCT04431024 |
| S28 | F | 66.1 | N | c.1938T>A | Stop gained | −0.1315452 | 36 | Pathogenic | P/LP | M1 | NCT04431024 |
| S29[a] | F | 29.9 | Y | c.1358_1359del | Frameshift | −0.1338676 | 26.2 | Pathogenic | P/LP | N/A | NCT04431024 |
| S30 | F | 37.8 | N | c.458delC | Frameshift | −0.1974506 | N/A | Pathogenic | P/LP | M0 | NCT04431024 |
| S31 | F | 60.4 | Y | c.592G>T | Stop gained | −0.1868802 | 36 | Pathogenic | P/LP | M3 | NCT04431024 |
| S32 | F | 58.3 | Y | c.102_109del | Frameshift | −0.207685896 | 33 | Pathogenic | P/LP | M1 | NCT04431024 |
| S33 | M | 50.6 | N | c.1938T>A | Stop gained | −0.1315452 | 36 | Pathogenic | P/LP | M2 | NCT04431024 |
| S34 | M | 32.8 | Y | c.510dupT | Frameshift | −0.183785775 | 29 | Pathogenic | P/LP | M2 | NCT04431024 |
| S35 | M | 39.8 | N | c.510dup | Frameshift | −0.183785775 | N/A | Pathogenic | P/LP | M1 | NCT04431024 |
| S36[a] | F | 43.2 | N | c.1717delC | Frameshift | −0.038271807 | 32 | Pathogenic | P/LP | N/A | NCT04431024 |
| S37 | M | 59.2 | N | c.510dup | Frameshift | −0.183785775 | N/A | Pathogenic | P/LP | M2 | NCT04431024 |
| S38 | M | 72.7 | N | c.1174C>T | Stop gained | −0.1915433 | 38 | Pathogenic | P/LP | M2 | NCT04431024 |

(continued)

# CADD Score Metrics

- A CADD score of 15 is the recommended minimum cutoff to identify deleterious mutations (Kircher *et al*., 2014; Rentzsch *et al*., 2019; 2021)

- A CADD score of 20 indicates that a variant is among the top 1% of the most deleterious variants in the human genome (Kircher *et al*., 2014; Pastorino *et al*., 2018)

- A CADD score of 30 places a variant in the top 0.1% of the most deleterious variants (mutations) in the human genome (Kircher *et al*., 2014; Rentzsch *et al*., 2019; 2021)

# Data/Evidence from Key Recent Studies

# Congedo 2024 – 20% estimate for genomic mesotheliomas

- "The incidence of MPM cases without an apparent association with asbestos exposure has been increasing in recent years, suggesting that genetic predisposing factors may play a crucial role."

- "Several candidate genes have been associated with a predisposition to MPM and most of them play a role in DNA repair mechanisms: overall, approximately 20% of MPM cases may be related to genetic predisposition."

- " "MPMs in these patients are significantly less aggressive, and patients require a multidisciplinary approach that involves genetic counseling, medical genetics, pathology, surgical, medical, and radiation oncology expertise.

Congedo MT, West EC, Evangelista J, et al. The genetic susceptibility in the development of malignant pleural mesothelioma: somatic and germline variants, clinicopathological features and implication in practical medical/surgical care: a narrative review. J Thorac Dis. 2024;16(1):671-687.

# Prassas 2025 - Pathogenic Germline BRCA2 Mutations Cause Mesothelioma and Other Cancers

- "Carriers of *BRCA2* PVs ($N = 1629$) had a significantly increased risk for four core HBOC-associated cancers (breast, ovarian, pancreatic, and prostate) and six additional types of cancer (lung, oral, small intestine, larynx, liver, and mesothelioma), hazard ratio (HR) > 2.37, all *p*s < 0.001."

- "Additionally, carriers were significantly associated with increased risks for six other cancer types, including cancer of larynx (HR = 3.89), liver (HR = 3.17), lung (HR = 2.69), mesothelioma (HR = 4.17), oral (HR = 2.80), and small intestine (HR = 3.90), all *p*s < 0.001."

- "Furthermore, overwhelming evidence in the last two decades suggests that the penetrance of cancer is also influenced by a large number of common cancer-specific risk-associated genetic variants in the genome [21]."

  - Prassas B, Shi Z, Tran H, et al. Estimating Cancer Penetrance in Carriers of BRCA2 Pathogenic Variants Using Cancer-Specific Polygenic Scores. *Cancer Med*. 2025;14(11):e70990. doi:10.1002/cam4.70990

# Wu (NCI - Schrump) 2025

- Published in July 2025, the study presents data gathered from a National Cancer Institute clinical trial to determine the ”natural history” of mesotheliomas in carriers of germline BAP1 mutations
- “A total of 50 patients with 32 unique BAP1 mutations were accrued to this protocol between March 2021 and July 2024 with data collection to August 31, 2024.”
- “The cohort included 24 males and 26 females with a median age of 51.2 years (range, 29.9– 73.3 y).”
- “Asbestos exposures, which traditionally have been difficult to measure and link consistently to prognosis of patients with mesothelioma, 16–18 were not objectively quantified in these subjects.”

# More Wu - Widespread "Multi-Compartment, Synchronous" Mesotheliomas Were Found Using Minimally Invasive Procedures

- The following data further proves genetic causation, and can be used to support requests for genetic testing data and to shape discovery.

- "Overall, of 43 subjects who underwent surgical evaluation of more than one cavity, 34 (79%) were found to have synchronous, subclinical multicompartment mesotheliomas (Supplementary Table 4A).

- Moreover, 14 of 15 subjects (93%) with a prior history of mesothelioma were found to have additional disease (26 of 29 compartments evaluated [90%]).

- In contrast, of 30 patients with no prior diagnosis of mesothelioma, 25 (83%) were found to have diffuse mesotheliomas at surgical evaluation, including 64 of 84 cavities (76%)."

# Genetic Findings from Wu (NCI - Schrump) 2025

- The following data further show genetic causation independent of asbestos fibers

- "Our findings clearly demonstrate that germline BAP1 mutations induce nonrandom alterations in DNA methylation with common and mutation-specific hotspots distributed across virtually every chromosome; presumably, these perturbations contribute to an increased risk of developing mesotheliomas and other tumors in subjects with BCS.

- Our DNA methylation analyses represent the most extensive, systematic evaluation of the epigenetic effects of naturally occurring germline BAP1 mutations in human cells and extend recent findings pertaining to potentially deleterious effects of CRISPR-mediated BAP1 mutations in HAP1 cells."

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KTH comment: In brief, HAP1 cells are widely used for genetic screening and functional genomic studies due to having a single copy of most genes, which quickly expose the effects of mutation. See Llargués-Sistac G, Bonjoch L, Castellvi-Bel S. HAP1, a new revolutionary cell model for gene editing using CRISPR-Cas9. *Front Cell Dev Biol*. 2023;11:1111488.

# More Wu - Results from Imaging Were Much Less Informative

- "In this study, we evaluated whether high-resolution CT imaging techniques in conjunction with VATS and DL, could facilitate detection of mesotheliomas in subjects with germline BAP1 mutations to gain information related to the prevalence and natural history of these neoplasms.

- "Our analysis revealed the presence of previously undiagnosed, multifocal mesotheliomas in the chest and/or abdomen in nearly 90% of subjects and more than 80% of all cavities examined; the vast majority of these mesotheliomas had no clearly discernible abnormalities on CT scans due to the small size and sessile nature of lesions.

- These findings strongly suggest that CT scans have limited utility in either detecting or ruling out early stage mesotheliomas in subjects with BCS.

- Furthermore, positron emission tomography/CT imaging does not appear to be a useful screening modality for these malignancies; of 105 previously undiagnosed compartments, only five (4.7%) displayed FDG avidity potentially suspicious for mesothelioma (data available on request).

# Krevanko 2025 - Genetics and Epidemiology Intersect

- **Abstract: "Conclusions:** Our findings suggest that a genetic predisposition for malignancy contributes to U.S. mesothelioma rates and is a distinct risk factor independent of asbestos exposure."

- Krevanko CF, Hernandez AM, Gauthier AM, Vahora MS, Lewis RC, Pierce JS. Potential influence of cancer history on mesothelioma incidence: an ecologic analysis in the U.S. population.

  - Krevanko CF, Hernandez AM, Gauthier AM, Vahora MS, Lewis RC, Pierce JS. Potential influence of cancer history on mesothelioma incidence: an ecologic analysis in the U.S. population. Krevanko CF, Hernandez AM, Gauthier AM, Vahora MS, Lewis RC, Pierce JS. Potential influence of cancer history on mesothelioma incidence: an ecologic analysis in the U.S. population. *J Public Health (Oxf)*. Krevanko CF, Hernandez AM, Gauthier AM, Vahora MS, Lewis RC, Pierce JS. Potential influence of cancer history on mesothelioma incidence: an ecologic analysis in the U.S. population. *J Public Health (Oxf)*. Published online September 7, 2025. doi:10.1093/pubmed/fdaf110

# Krevanko 2025 - Note Especially the Statistics for Females

- **Abstract: "Conclusions:** Our findings suggest that a genetic predisposition for malignancy contributes to U.S. mesothelioma rates and is a distinct risk factor independent of asbestos exposure."

- "Approximately 25% (n=3969) of all female mesothelioma cases were diagnosed with another primary cancer during the 20-year study period."

- "Among females with another primary cancer diagnosis, mesothelioma was recorded as the second of two or more malignancies in 68% of females and the third of three or more malignancies in~14%off females (Table2)."

# Carbone 2025 - More Evidence that Some BAP1 Mutations Cause Mesotheliomas by Themselves

- "We identified 34 different germline inactivating mutations."
- "Among 238 BAP1$^{+/-}$ carriers aged 27-81, 84 were diagnosed with mesothelioma (35%), 1/84 had evidence of asbestos exposure."
- "No mesothelioma was recorded among 123 siblings/relatives who did not inherit BAP1$^{+/-}$ $p<0.0001$."
  - Carbone M, Minaai M, Kittaneh M, et al. Clinical and Pathologic Phenotyping of mesotheliomas developing in carriers of Germline BAP1 Mutations. *J Thorac Oncol*. doi:10.1016/j.jtho.2025.06.020

# Nielsen 2025 – Null Variant Germline BAP1 Mutations Can Cause Mesothelioma by Themselves - Dr. van Zyl and Colleagues

- Overall, the authors concluded that the probability that malignant mesothelioma in *BAP1* mutant mice would be highly unlikely if *BAP1* mutations did NOT cause Mesothelioma. Specifically, based on the data, the authors concluded that the likelihood is approximately 97.9% certain that the rate of mesothelioma in *BAP1* mutant mice occur at a minimum of twice the rate of mesothelioma in wild type mice.

- *"Our analysis concurs with the biological data showing that pathogenic germline BAP1 mutations are sufficient to cause malignant mesothelioma independent of external factors (i.e., exposure to asbestos). This now eliminates the apparent contradiction between the published literature on biological findings showing the impact of variants in highly penetrant tumor suppressor genes, in this case specific BAP1 variants, and the lack of findings of the same effect in mouse studies with non-exposed subjects having those same genetic variants."* Nielsen et al., 2025

  - Nielsen DM, Hsu M, Zapata M 3rd, Ciavarra G, van Zyl L. Bayesian analysis of the rate of spontaneous malignant mesothelioma among BAP1 mutant mice in the absence of asbestos exposure. *Sci Rep*. 2025;15(1):169. doi:10.1038/s41598-024-84069-w

# Febres-Aldana 2023 – Germline Variants Cause Mesothelioma

- Physicians and researchers from Memorial Sloan Kettering Cancer Center (MSKCC) issued a notable fall 2023 article with the following three key statements:

- "Other causes unrelated to asbestos are being more clearly defined including other nonasbestos minerals, germline variants, etc." at 12

- "Moreover, biallelic BAP1 inactivation has been shown to be sufficient to drive the development of nonasbestos-related mesothelioma (85) and is synergistic with loss of NF2 or CDKN2A in inducing mesothelioma in mice (97)." at 19

- "Remarkably, while BAP1 inactivation is oncogenic, not all BAP1-deficient DPMs exhibit poor prognosis. A subset of epithelioid DPMs arising in the context of germline BAP1 mutations is less aggressive, showing significantly improved survival (92)." at 20

Febres-Aldana, C. A., Fanaroff, R., Offin, M., Zauderer, M. G., Sauter, J. L., Yang, S. R., & Ladanyi, M. (2024). Diffuse Pleural Mesothelioma: Advances in Molecular Pathogenesis, Diagnosis, and Treatment. *Annual review of pathology*, *19*, 11–42.

# Some of the Many Take Aways from the Data in Key Recent Studies

- Make sure to think beyond BAP1, and think even more about BRCA1 and 2
- Find out if plaintiff is part of the NCI BAP1 study reported in Wu, and obtain all medical records from NCI
- Wu findings suggest parties should agree to more autopsies that may reveal multi-compartmental mesothelioma, thereby suggesting/revealing genetic causation

# Germline Mutations Are Not Predictable

# Cheung 2021 (Testa Lab) – Impossible to Predict Variants in Each Person



**Table 2.** Variants observed in germline DNA from 14 MM patients

| Patient | Gene(s) | Variant(s) | Predicted Protein Change(s) | Type of variant(s) | ExAC_All %; CADD score |
|---|---|---|---|---|---|
| ABS2406 | *MSH4* | NM_002440.4:c.719dupT | NP_002431.2:p.Ile240fs | Indel | Novel; 35 |
| ABS2640 | *CHEK2* | NM_007194:c.909-2028_1095+330del5395 | NP_009125.1:p.M304Lfs*16 | Del. (exons 9-10) | Novel; NA |
| | *DACT2* | NM_214462.3:c.533C>T | NP_999627.2:p.Ser178Leu | Missense | Novel; 25.9 |
| | *LRRK2* | NM_198578.3:c.6055G>A | NP_940980.3:p.Gly2019Ser | Missense | 0.0004; 32 |
| ABS2813 | *MUTYH* | NM_001128425.1:c.389-1G>A | Frameshift / truncation | Splice site | Novel; 33 |
| ABS3425 | *DNMT3A* | NM_022552.4:c.2631delC | NP_072046.2:p.Ser689fs | Indel | Novel; 35 |
| | *POLE4* | Whole gene deletion | No protein expression | Whole gene del. | Novel; NA |
| | *APC* | NM_000038.5:c.1642T>G | NP_000029.2:p.Leu548Val | Missense | Novel; 22.7 |
| ABS3460 | *POLQ* | NM_199420.4:c.3589C>T | NP_955452.3:p.Arg1197* | Nonsense | 1.65E-05; 35 |
| | *XRCC1* | NM_006297.2:c.175delG | NP_006288.2:p.Asp59fs | Indel | 8.24E-06; 35 |
| | *SETD1B* | NM_015048.1:c.2554C>T | NP_055863.1:p.Arg852Cys | Missense | 5.11E-05; 24 |
| | *ARID1B* | NM_017519.2:c.2405C>T | NP_059989.2:p.Ser802Leu | Missense | 0.0004; 32 |
| 946-P | *BRCA2* | NM_000059.3:c.657_658del | NP_000050.2:p.Thr219fs | Indel | 6.12E-05; 22 |
| | *LRRK2* | NM_198578.3:c.5314_5317+6delAAAGGTAAGG | Frameshift/truncation | Indel at splice site | Novel; 35 |
| R88-T | *LRRK2* | NM_198578.3:c.5314_5317+6delAAAGGTAAGG | Frameshift/truncation | Indel at splice site | Novel; 35 |
| | *ATM* | NM_000051.4:c.1837G>T | NP_000042.3:p.Val613Leu | Missense | 4.26E-05; 25.5 |
| ABS3383 | *CHEK2* | NM_007194:c. 1451C>T | NP_009125.1:p.Pro484Leu | Missense | 0.0001; 29.7 |
| | *POLE* | NM_006231.2:c.177G>C | NP_006222.2:p.Lys59Asn | Missense | 4.12E-05; 21.7 |
| ABS3481 | *ATR* | NM_001184.4:c.4351C>T | NP_001175.2:p.Arg1451Trp | Missense | 0.0003; 26 |
| | *JARID2* | NM_004973.4:c.3682G>A | NP_004964.2:p.Val1228Met | Missense | 6.59E-05; 23.7 |
| ABS3505 | *POLQ* | NM_199420.4:c7024_7026TAA>AAT | NP_955452.3:p.Leu2342Ile | Missense | 0.0002; 24.2 |
| | *BRIP1* | NM_032043.3:c.2220G>T | NP_114432.2:p.Gln740His | Missense | 0.0005; 23.7 |
| | *CBFA2T3* | NM_005187.5:c.956G>A | NP_005178.4:p.Cys319Tyr | Missense | 1.76E-05; 23.7 |
| | *RHBDF2* | NM_024599.5:c.1726A>G | NP_078875.4:p.Ile576Val | Missense | 2.02E-05; 21.6 |
| ABS3572 | *MLH3* | NM_001040108.2:c.G3455G>A | NP_001035197.1:p.Arg1152His | Missense | 6.59E-05; 24.1 |

# Flexible Reporting of Sequencing Results Can Eliminate Inefficiencies and Many Disputes

# Flexible Reporting of Sequencing Results Can Reduce Inefficiencies and Disputes

- Disputes over the scope of and reporting of sequencing results really should not arise because Dr. Testa and others have proved and testified that it is not possible to predict which genes carry germline mutations or which mutations are present
  - nonetheless, time wasting and economically inefficient disputes sometimes are generated by lawyers

- To reduce disputes and waste, we worked with a small CLIA certified sequencing lab in California to develop a flexible sequencing results reporting process

- To avoid wastes of time and/or money for litigants and courts, the lab will run whole genome sequencing (WGS) so that the best possible and complete sequencing data is captured and stored

- The lab, however, is smart and flexible, and so will initially report sequencing results only for the set of genes agreed to by the parties or ordered by a judge

- Subsequently, the lab will report additional sequencing results if agreed to by the parties or ordered by a judge

# Issues re Genetic Data Secrecy

# Defense Losing Data/Evidence Because of Genetic Secrecy Orders

- Plaintiff firms often demand and obtain stipulations or orders that:
  - limit disclosure of genetic data in a particular case
  - preclude use of a case's genetic data in other cases
  - preclude use of genetic data in articles to be submitted to peer-reviewed journals by defense-retained experts, unless plaintiff consents
- Secrecy orders with such terms are precluding defendants from aggregating additional evidence of genomic causation

# Genomic Evidence: Focus on Objectivity and Reproducibility

# Plaintiff Expert Reliance on 2 Hit Theory is Not Reliable

- Multiple experts called by plaintiffs (e.g. Zhang, Brody, Staggs, Kradin, Felsher, Testa, have relied on Knudson's 2 hit theory of cancer to blame asbestos for causing mesotheliomas and other cancers
- Knudson's 2 hit theory is over 50 years old
- Multiple peer reviewed studies over the last 25 years have proved there are numerous exceptions to the 2 hit theory (e.g. Chernoff 2021)
- Most defense counsel do not – but should - challenge reliability of 2 hit testimony by plaintiff experts

# Recent Stipulations and Orders for Extensive, Flexible Sequencing

# Extensive WGS and Flexible Sequencing Stipulations in Mizer and Kudenov Cases

- Two mesothelioma cases (Mizer in NJ and Kudenov in CA) recently resulted in stipulations to extensive genetic sequencing, including flexible initial and subsequent reporting of sequencing results

- Both stipulations result in whole genome sequencing (WGS), meaning sequencing of the entirety of all genes
  - WGS is important for multiple reasons, including:
    - because Dr. Testa and others have proved that it is not possible to predict which genes carry germline mutations or which mutations are present
    - exome sequencing fails to cover about 98% of each gene
    - only WGS reveals large deletions and other less common mutations

- Both stipulations result in initial reporting of extensive sequencing results:
  - over four hundred genes (Mizer)
  - over eighty genes (Kudenov)

- After initial results are reported, defendants have the option to seek disclosure of additional sequencing results
-

# The Plaintiff Did Not Timely Produce Genetic Testing Results in Both Mizer and Kudenov Cases

- Despite the frequent presence of genetic testing results in medical records for people with mesothelioma, the plaintiff did not timely produce genetic testing results in both the Mizer and Kudenov cases

- In Mizer, in August 2024, plaintiff's counsel (Maune Raichle) belatedly produced a Foundation Medicine genetic testing report dated May 2023
  - The report revealed, among others, the presence of a pathogenic *BAP1* mutation

- In Kudenov, in September 2024 (essentially at the start of trial), plaintiff's counsel belated produced a Foundation Medicine genetic testing report dated in February 2024
  - The report revealed, among others, the presence of a pathogenic *ERCC4* mutation

# Recent Rulings

- Numerous state courts have entered orders for genetic testing in cases involving birth defects, and cancers allegedly caused by asbestos, benzene, fungicides, drugs and other toxicants.

- Outside California, recent rulings granting defense motions for genetic testing include Florida (Maute), Hawaii (McCabe), Louisiana (Craft), Massachusetts (Gonzalez) and Washington (Beckwith).

- In California, notable recent rulings are Combs and Stark in Alameda.

# Missing Genetic Sequencing Results – "Family Matters" article

- All stakeholders should be aware of the following key quotes from the "Family Matters" article; the first author is a genetic counselor, F. Hathaway, from the University of Chicago.
  - May 2023 - Hathaway F, Martins R, Sorscher S, Bzura A, Dudbridge F, Fennell DA. Family Matters: Germline Testing in Thoracic Cancers. *Am Soc Clin Oncol Educ Book*. 2023;43:e389956.
  - Open access at https://ascopubs.org/doi/10.1200/EDBK_389956?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200pubmed

- "Many patients with mesothelioma are involved with litigation when it comes to their personal diagnosis of mesothelioma due to exposure to asbestos."

- "Many of these patients are rightfully concerned that if a germline mutation is identified, this can be used against them. That is, the defense could argue that the asbestos exposure had little to no effect on cancer diagnosis and that the cause of the cancer was due to an underlying predisposition to mesothelioma…."

- "It is imperative that genetic counselors discuss this potential with patients so that they can make the best decision for themselves regarding genetic testing. Viable alternative options may (1) include a decline in germline genetic testing or (2) choose to undergo genetic testing under a research study to keep this information separate from their medical record." (emphasis added)"

# More on Missing Genetic Sequencing Results - Follow Up "Family Matters" Talk at ASCO 2023

- ***The following text is from the ASCO-provided transcript of Q & A after the 2023 ASCO Family Matters panel presentation by Ms. Hathaway, Dr. Fennell and others:***

- "SPEAKER [3 Dr. Fennell]: …What do you do if you have experience of germline mutation in a particular patient affecting the likelihood of successful litigation?"

- "SPEAKER [1 Ms. Hathaway]: Yeah, so, so far, they haven't been able to have access to the testing itself. So interesting enough, at least in the United States, **this is why Dr. Kindler set up her research, mainly to avoid having to disclose that when they ask for health records**."

- "…which is why I would say that the majority of my mesothelioma patients that we see when they come back positive under the research study, **I've yet to have anybody want to do clinical-based testing after getting--knowing that they have their mutation research-wise, because we don't put it in the medical record at that point**."

- **"We do talk to the patient's family members. We encourage them to call us and tell--and go over what does this mean."**

- *Video and transcript are available to ASCO members at the following link: https://meetings.asco.org/2023-asco-annual-meeting/15123?presentation=215121#215121*

# Limits of Exome Sequencing Reports and "Actionability" Reports

# Exome Tumor Sequencing Misses About 98% of Each Gene, and Does Not Rule Out Other Mutations

- Exome sequencing gene "panels" have inherent limitations:
  - they do no detect variants in deep intronic regions of genes, and do not detect large deletions, whole gene deletions, and large structural rearrangements.

- Somatic tumor sequencing does not rule out the possibility that a person carries pathogenic germline mutations

- These principles actually matter for mass tort cases
  - For example, multiple published peer reviewed studies have now shown that familial whole *BAP1* gene deletions do occur, including deletions of large sections of the *BAP1* gene (*i.e.,* Walpole *et al*., 2018; Boru *et al*., 2019; Carbone *et al*., 2022; Pandithan *et al*., 2022 etc.)

# "Actionable" and Clinically Significant Reports Do Not Rule the Presence of Pathogenic Mutations

- Do not be fooled by "actionable" or "clinically significant" reports of no mutations, such as below
- In brief, those reports are limited to mutations that can be treated with a precision medicine

***BRCA1/2 Analyses with CancerNext-Expanded®: Analyses of Genes Associated with Hereditary Cancer (90 genes)***

**RESULTS**

Print

Pathogenic Mutation(s): None Detected
Variant(s) of Unknown Significance: None Detected
Gross Deletion(s)/Duplication(s): None Detected

**SUMMARY**

**NEGATIVE: No Clinically Significant Variants Detected**

**INTERPRETATION**

- No pathogenic mutations, variants of unknown significance, or gross deletions or duplications were detected.
- **Risk Estimate:** low likelihood of variants in the genes analyzed contributing to this individual's clinical history.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

ToxicoGenomica

# Genetically-Caused Mesotheliomas ≠ Asbestos-Induced Mesotheliomas

## Clinical, morphologic, epidemiologic, and biologic distinctions

**Len van Zyl, Ph.D.**

**1 October 2025**

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# Overall Conclusions #1

- **Strong clinical evidence that genetically-driven mesotheliomas represent a distinct disease entity (Wu *et al*., 2025; Carbone *et al*., 2025)**
- Wu *et al*., (2025) clinical study examined 50 people who carry 32 unique pathogenic germline *BAP1* mutations; 70% of the patients presented with prior *BAP1* cancers.
- Wu (2025) and Carbone (2025) data shows that germline *BAP1*-driven MM tumors are bi-lateral and/or multi-compartmental tumors and occurred in the majority of the study members
  - Multicompartment disease: majority of patients had synchronous pleural + peritoneal mesotheliomas; many had tri-compartment involvement. Synchronous, bi-lateral tumors are **"RED FLAGS"** for genetically caused disease
  - Majority of *BAP1* germline carriers had subclinical mesotheliomas despite minimal or no CT findings.
  - **Lesions are multicompartmental, low-grade, and indolent, not resembling classic asbestos-driven mesothelioma**
  - **Genomic analyses revealed that pathogenic germline *BAP1* mutations induce non-random somatic mutations, including epigenetic changes** (*i.e.*, non-random changes in methylation patterns)
- **Clinical strategy**: observation + surveillance, not immediate therapy

# Overall Conclusions #2

- *Genetic predisposition for U.S. mesothelioma rates is a distinct risk factor independent of asbestos exposure* (Krevanko *et al*., 2025)
- In the context of Malignant Mesothelioma (MM), my group and I recently published a study in Nature Scientific Reports (Nielsen *et al*., 2025) confirming that highly penetrant pathogenic germline mutations in the *BAP1* Gene in mice can and do cause mesothelioma independently of asbestos exposure.
- ***"It is very important that those caring for these patients understand that genetically linked mesotheliomas, especially when detected at an early stage, have a much less aggressive clinical course compared to patients with asbestos-induced mesotheliomas: These are different diseases. The former is minimally invasive, patients survive for several years and respond to therapy. Some patients have been cured..."*** Novelli *et al*., 2024

# Overall Conclusions #3

## Germline Variants Directly Drive (Determine) Subsequent Tumor Characteristics

- Substantial evidence (from NGS experiments of extremely large populations) are showing that the subsequent acquired somatic (tumor) mutations are NOT RANDOMLY AQUIRED [via stochastic replication errors (R) alone]; or as we age;
- Rather, they are PRE-DETERMINED to a large extend by Inherited (germline) Pathogenic or Polymorphic Variants with direct health consequences
- Also importantly, new data supports a direct link between germline variants and immune evasion or suppressed anti-tumor response during cancer progression
- Numerous large scale research studies have now unequivocally shown that specific germline variants in the genome (coding or non-coding) impact which somatic events and mutations are generated and selected for in cancer cells during tumorigenesis
- Germline pathogenic variants play a significant role in cancer; including the immune system, highlighting that heredity does not play a minimal, but a dominant role in cancer tumorigenesis

# Krevanko (September 7, 2025) – Genetic Predisposition is an Asbestos Independent Risk Factor for Mesothelioma

*"Our findings suggest that a genetic predisposition for malignancy contributes to U.S. mesothelioma rates and is a distinct risk factor independent of asbestos exposure."* Krevanko *et al.*, 2025

Journal of Public Health | pp. 1–6 | https://doi.org/10.1093/pubmed/fdaf110

## Potential influence of cancer history on mesothelioma incidence: an ecologic analysis in the U.S. population

**Callan F. Krevanko[1], Ashley M. Hernandez[2], Alison M. Gauthier[3], Moin S. Vahora[2], Ryan C. Lewis[3,*] and Jennifer S. Pierce[2]**

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**ABSTRACT**

**Background:** There is a demand for population level research on the potential genetic-basis of mesothelioma (e.g. BRCA1-associated protein-1 [BAP1]) independent of other risk factors, such as amphibole asbestos exposure. By surrogate, another primary cancer history can be used to explore this issue, including in the USA, where the incidence rates (IRs) in men, but not women, are temporally aligned with historical asbestos consumption.

**Methods:** We computed age-adjusted IRs of mesothelioma in females and males stratified by other primary cancer history using publicly available U.S. cancer data from 1975 to 2021. To facilitate comparison with other cancers associated with BAP1, we calculated age-adjusted IRs for female breast cancer and melanoma.

**Results:** Similar to breast cancer and melanoma, ~25% of females with mesothelioma had a history of at least one other primary cancer. While IRs of mesothelioma in males without a history of other primary cancers were temporally aligned with historical asbestos consumption trends in the USA, IRs of mesothelioma among males with other primary cancer histories showed no relationship with asbestos consumption trends.

**Conclusions:** Our findings suggest that a genetic predisposition for malignancy contributes to U.S. mesothelioma rates and is a distinct risk factor independent of asbestos exposure.

**Keywords:** mesothelioma; multiple primary neoplasms; risk factors; SEER program

### Introduction

In the USA, mesothelioma is a rather uncommon cancer, with an annual age-adjusted incidence rate (IR) of < 1 per 100 000 persons at risk.[1] Prior to the initial impacts of the coronavirus disease from the SARS-CoV-2 virus in 2020, mesothelioma accounted for ~0.10% of all mortality in the USA per year.[2] Mesothelioma bears a unique characteristic: incident cases in the USA occur in a relatively high percentage of individuals with a history of prior cancer of a different type.[3,4] For example, an analysis of data from the National Cancer Institute's (NCI) Surveillance, Epidemiology, and End Results (SEER) Program revealed that among incident mesotheliomas in 2009–2013, 10.9% of those aged 20–64 years and 23.5% of those aged ≥ 65 years had been previously diagnosed with cancer at another site.[3] For context, compared to 29 other incident cancers, the rank-order of these two percentages was one of 29 and two (tied) of 29, respectively. A more recent SEER analysis revealed that 18% of incident mesotheliomas in 2019 among those aged ≥ 18 years were among persons with a previous cancer at another site, corresponding to a rank-order of two of 29.[4]

Patients with a history of multiple primary cancers are more likely to have a hereditary predisposition to malignancy due to their increased probability of carrying germline mutations in cancer-related genes.[5,6] Accordingly, the International

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# MM Developing in *BAP1*$^{+/-}$ Carriers is a Different Disease: Biologically, Histologically, and Clinically

ORIGINAL ARTICLE

## Prospective Analysis of Mesotheliomas in Subjects With BAP1 Cancer Syndrome: Clinical Characteristics and Epigenetic Correlates of Disease


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**ABSTRACT**

**Introduction:** Although mesotheliomas are the most common malignancies identified in BAP1 cancer syndrome (BCS), the prevalence and natural history of the neoplasms have not been elucidated. Protocol NCT04431024 was initiated to prospectively evaluate whether high-resolution computed tomography (CT) imaging and minimally invasive surgical evaluation could facilitate detection and surveillance of mesotheliomas in subjects with germline *BAP1* mutations.

**Methods:** Subjects above or equal to 33 years of age with or without prior malignancies underwent CT imaging followed by bilateral thoracoscopies and laparoscopies. CT imaging and intraoperative findings were objectively scored; surgical biopsies were interpreted by two expert pathologists. Skin, peripheral blood mononuclear cells, plasma, serum, and tumor biopsies were collected for correlative research studies.

**Results:** A total of 50 subjects with 32 germline *BAP1* mutations were enrolled between March 2021 and July 2024. Median follow-up was 21.8 months (range: 1.7–41.1 mo). Furthermore, 16 sites of prior mesothelioma in 15 patients were excluded from the analysis. Surgical



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Journal Pre-proof

## Clinical and Pathologic Phenotyping of mesothelioma developing in carriers of Germline *BAP1* Mutations

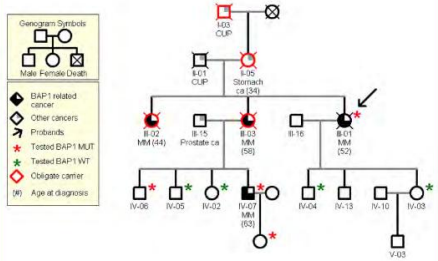
Journal of Thoracic Oncology

### Introduction - The *BAP1* Cancer Syndrome and Mesothelioma (MM)

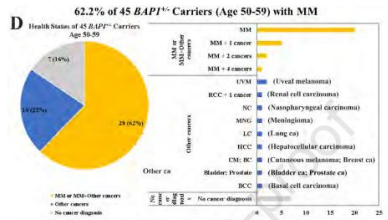
We discovered that familial MM was transmitted in a Mendelian fashion[1]; that germline *BAP1* mutations caused MM[2] and other cancers[3]; and we elucidated mechanisms of BAP1 activity.[4-6]

Here we present our findings studying from 1999-2024, 47 families carrying heterozygous germline *BAP1* mutations (*BAP1*$^{+/-}$), including 238 *BAP1*$^{+/-}$ carriers and 123 *BAP1*$^{+/+}$ relatives.

**Representative Family Tree**

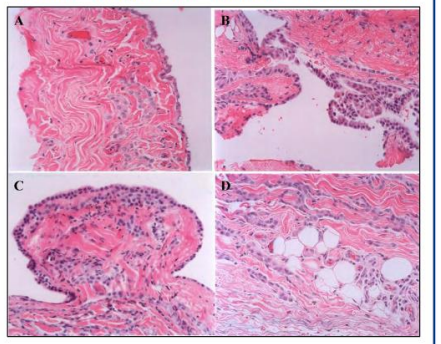



### Results




There were 84 cases of MM and 22 cases of UVM among the 238 *BAP1*$^{+/-}$ carriers, 8 of them developed both malignancies. None of the 123 first and second-degree relatives with WT *BAP1*$^{+/+}$ were diagnosed with MM [$\chi^2(1) = 56.6$, $p < 0.0001$], or with UVM [$\chi^2(1) = 92.1$, $p < 0.0001$].

### MM in *BAP1* mutant carriers is a different disease compared to asbestos induced MM




Characteristic Histology (A-C), most patients have three-cavitary diffuse mesothelial hyperplasia and (D), focal areas of low-grade germline-mutant-*BAP1*-associated mesothelioma (L-BAM). L-BAM are superficially invasive mesothelial neoplasms that show BAP1 loss. Most microscopic L-BAMs do not progress, if they become visible on imaging these neoplasms should be removed. This patient was treated with extrapleural pneumonectomy at age 43 and died 19.5 years later of a stroke. MM never recurred.

**CONCLUSION:** Compared to sporadic MM, MM developing in *BAP1*$^{+/-}$ carriers are a different disease, biologically, histologically and clinically: these patients require a tailored clinical approach. Because these patients are at high risk of developing multiple cancers, they benefit from annual screening for early cancer detection that can be life-saving.

IASLC

**Carbone M, Minaai M, et al. *J Thorac Onc* (2025)**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Two Independent Studies Uncover a Novel, Less Aggressive Variant of Mesothelioma Linked to Germline *BAP1* Mutations (Wu 2025-National Cancer Institute (NCI); Carbone 2025; University of Hawaii Cancer Center— illuminate a previously unrecognized subset of mesothelioma patients whose tumors exhibit markedly less aggressive clinical behavior and respond favorably to therapeutic interventions.

# NCI's Wu 2025 Study Conclusions

- *"Although no direct comparison was performed, our prospective findings (including our recent retrospective analysis[35]) **strongly suggest that mesotheliomas arising in subjects with germline BAP1 mutations are distinctly different from sporadic mesotheliomas with or without somatic BAP1 mutations**.[10,36]"*

- ***"Whereas sporadic mesotheliomas predominantly involve one body cavity,[37] germline BAP1 mutant mesotheliomas arise as synchronous, multicompartment malignancies; our next-generation sequencing studies (Gilson et al., unpublished data, 2025) confirm that these lesions are polyclonal, consistent with a field cancerization phenomenon."***

- *"Although lead-time bias[38] cannot be ruled out,* ***rates of progression and lethality of early stage mesotheliomas arising in subjects with BCS appear to be slower than those of sporadic mesotheliomas[36]; in all likelihood, the unique molecular and histologic phenotypes of germline BAP1 mutant mesotheliomas described in this study contribute to significantly prolonged survivals of patients with these neoplasms versus those with more common, sporadic mesotheliomas.[10,36]"*** Wu *et al*., 2025

# NCI's Wu 2025 Study Conclusions

- *"**Our experience to date indicates that diffuse, subclinical mesotheliomas in subjects with BCS do not warrant immediate interventions, such as surgery, immuno-therapy, or chemotherapy, as patients with these malignancies may live for extended periods before manifesting clinically evident disease**."*

- *"We recommend periodic surveillance, as outlined in Supplementary Table 2, with standard-of-care interventions once disease is apparent on conventional imaging studies or there is rapid progression on minimally invasive surgical assessment; all surveillance and interventions in subjects with BCS should be per-formed in the context of prospective clinical trials."*

- ***"Close multidisciplinary follow-up is essential for these subjects, given their propensity to develop other cancers and the potential for their relatively indolent mesotheliomas to evolve into lethal malignancies**; it is important for clinicians and carriers of germline BAP1 mutations to recognize these risks and realize that, presently, there are no validated biomarkers that can predict which mesotheliomas will become life threatening."* Wu *et al*., 2025

# NCI's Wu 2025 Study Conclusions

- ***"Our DNA methylation analyses represent the most extensive, systematic evaluation of the epigenetic effects of naturally occurring germline BAP1 mutations in human cells and extend recent findings pertaining to potentially deleterious effects of CRISPR-mediated BAP1 mutations in HAP1 cells.[28]***

- ***Our findings clearly demonstrate that germline BAP1 mutations induce nonrandom alterations in DNA methylation with common and mutation-specific hotspots distributed across virtually every chromosome; presumably, these perturbations contribute to an increased risk of developing mesotheliomas and other tumors in subjects with BCS."*** Wu *et al*., 2025

- ***"Our findings provide preliminary proof-of-concept that systemic epigenetic burdens in normal, non-target tissues are linked to cancer predilection in subjects with BCS."*** Wu *et al*., 2025

# The Direct Impact of Germline Variants On Somatic Events During Tumorigenesis

- Cancer is characterized by diverse genetic alterations in both germline and somatic genomes that disrupt normal biology and provide a selective advantage to cells during tumorigenesis (Ramroop *et al*., 2019).
- Analyses integrating data from both germline and somatic genomes for the same person have identified numerous germline variants that directly impact somatic events in tumors, including inducing hotspot driver mutations.
- **These interactions between specific germline variants help determine/influence cancer subtypes, treatment response, and clinical outcomes** (Ramroop *et al*., 2019).
- In short: ***Germline genetic variants play a critical role in shaping the selection of and generation of specific somatic mutations during tumorigenesis*** (Ramroop *et al*., 2019).

As demonstrated by the Wu *et al*., 2025 germline *BAP1* study

# Germline Variants Directly Impact Tumor Characteristics

- Using next-generation sequencing, the germline and tumor genomes have been explored independently for mutations that are associated with tumorigenesis, often with the germline genome serving as a reference control for acquired (somatic) mutations (Ramroop *et al*., 2019; Srinivasan *et al*., 2021; Vali-Pour *et al*., 2022; *etc.*)
- Analyses integrating data from both germline and somatic genomes of the same person have led to the discovery of novel associations between germline variants and specific somatic events.
- ***Research have now unequivocally shown that specific germline variants impact which somatic events and mutations are generated and selected for in cancer cells during tumorigenesis*** *(*Ramroop *et al.,* 2019*)*

# The Context-Specific Role of Germline Pathogenicity in Tumorigenesis

- **Srinivasan *et al*. at Memorial Sloan Kettering Cancer Center published a study in 2021 which demonstrated how pathogenic germline mutations shape the somatic landscape to promote cancer initiation and progression.** The authors leveraged a unique, pan-cancer clinical cohort of matched tumor and normal genomic data (*i.e.*, 17,152 cancer patients diagnosed with 55 broad cancer types and 413 histological subtypes; matched germline and tumor DNA were used to sequence 468 cancer-associated genes)

- They showed that carriers of high penetrance pathogenic variants, lineage-dependent selective pressure for biallelic inactivation ***was associated with earlier age of onset and specific somatic phenotypes indicative of dependence on the germline allele for tumorigenesis*** (Srinivasan *et al*., 2021).

- ***"In such patients, this germline "driver" was likely the founding event that directly promoted cellular transformation and tumor initiation, ultimately shaping the somatic mutational profile of the resulting tumors, with subsequent somatic driver events arising to accelerate tumor formation, progression, and potentially therapeutic sensitivity and resistance"*** **Srinivasan *et al*., 2021**

# The Context-Specific Role of Germline Pathogenicity in Tumorigenesis

- ***"These results have wide-ranging implications for the clinical management of cancers arising in carriers of pathogenic cancer-associated variants."***
- ***"Ultimately, they signal the need for a fundamental shift in current approaches to clinical assessment, whereby an integrated analysis of somatic and germline alterations is required in order to present a more complete view of a patient's cancer."***
- ***"In this framework, somatic features such as biallelic inactivation and co-occurring mutational signatures in the arising tumor complement population frequency and family history to directly inform the interpretation of germline variants."***

Srinivasan *et al.*, 2022

# Highly Penetrant Pathogenic Germline Variants In Essential Genes Drive Tumorigenesis Without Other Factors

GERMLINE MUTATIONS

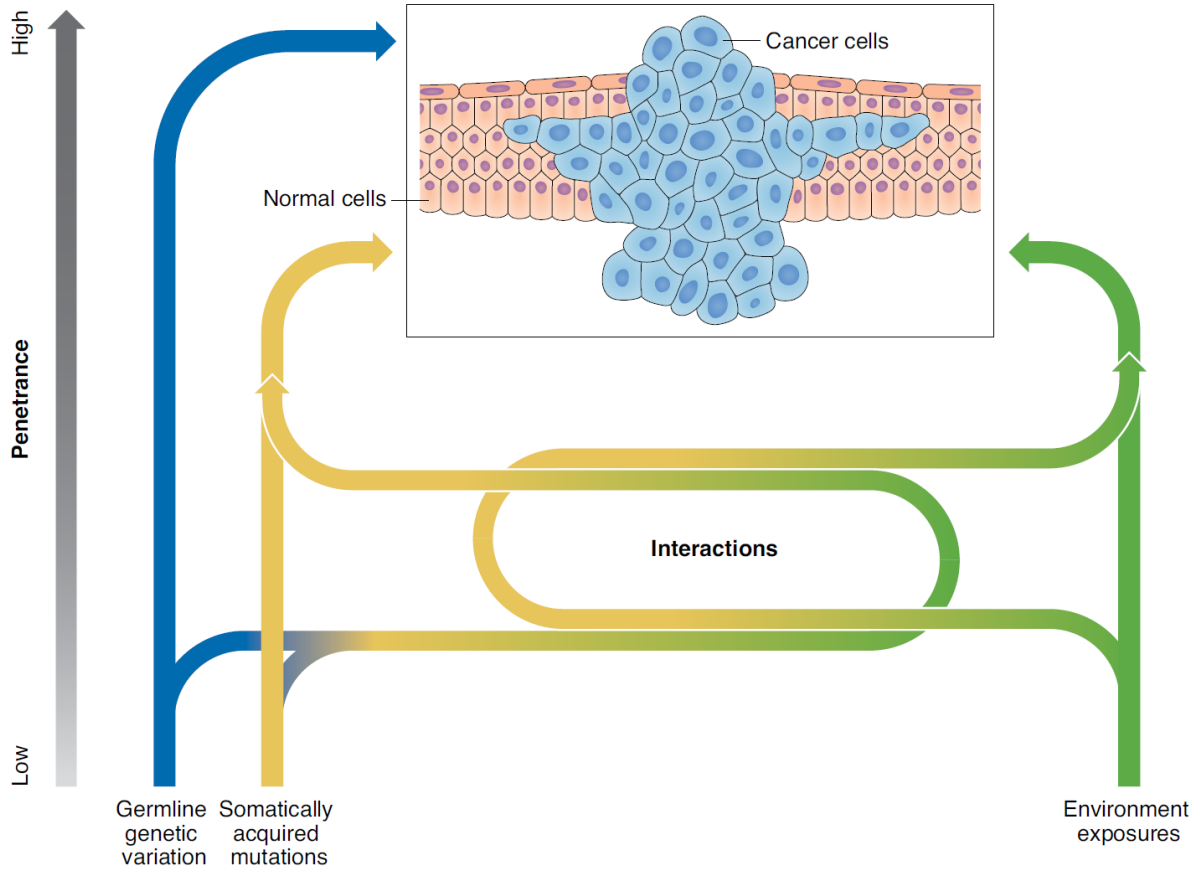
## How the germline informs the somatic landscape

How somatic and germline mutations interact in cancer remains largely unexplored. A study of 17,152 patients with cancer suggests that the relative contribution of pathogenic germline mutations is governed by lineage and penetrance.

Stephen J. Chanock

**NATURE GENETICS** | VOL 53 | NOVEMBER 2021 | 1522–1526 | www.nature.com/naturegenetics

# Highly Penetrant Pathogenic Germline Variants In Essential Genes Drive Tumorigenesis Without Other Factors




## Dynamic Model of Carcinogenesis:

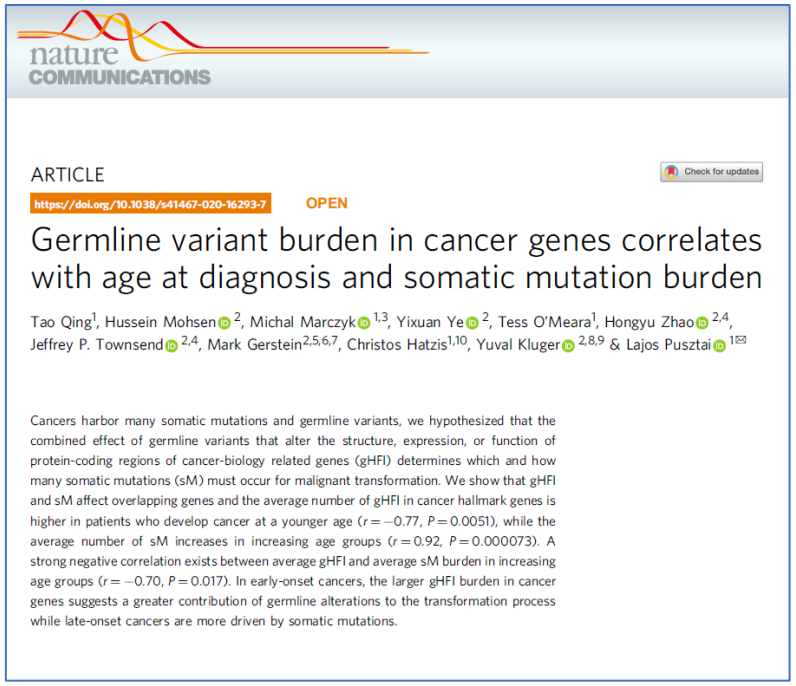
*"Tumor development depends on complex interactions between germline variants (dark blue), somatically acquired variants (yellow) and environmental exposures (green).* ***Penetrance also has a modifying role in which highly penetrant (germline) variants are more likely to drive tumorigenesis without the influence of other factors."***

Chanock, 2021; Nature Genetics

Adapted from Chanock, 2021; based on data generated from a massive genomics study by Srinivasan *et al.*, 2021; Nature Genetics

# Deleterious Germline Mutations Drive Tumor (Somatic) Characteristics

## Multiple Similar Large Scale Genomic Studies Arrived at the Same Conclusion



nature COMMUNICATIONS

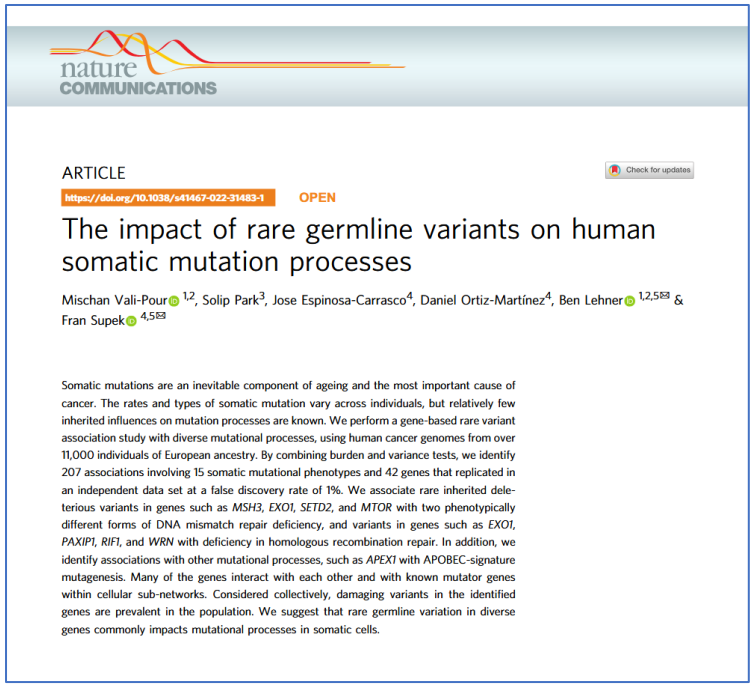
ARTICLE

https://doi.org/10.1038/s41467-020-16293-7 OPEN

Germline variant burden in cancer genes correlates with age at diagnosis and somatic mutation burden

Tao Qing[1], Hussein Mohsen[2], Michal Marczyk[1,3], Yixuan Ye[2], Tess O'Meara[1], Hongyu Zhao[2,4], Jeffrey P. Townsend[2,4], Mark Gerstein[2,5,6,7], Christos Hatzis[1,10], Yuval Kluger[2,8,9] & Lajos Pusztai[1]

Cancers harbor many somatic mutations and germline variants, we hypothesized that the combined effect of germline variants that alter the structure, expression, or function of protein-coding regions of cancer-biology related genes (gHFI) determines which and how many somatic mutations (sM) must occur for malignant transformation. We show that gHFI and sM affect overlapping genes and the average number of gHFI in cancer hallmark genes is higher in patients who develop cancer at a younger age ($r = -0.77$, $P = 0.0051$), while the average number of sM increases in increasing age groups ($r = 0.92$, $P = 0.000073$). A strong negative correlation exists between average gHFI and average sM burden in increasing age groups ($r = -0.70$, $P = 0.017$). In early-onset cancers, the larger gHFI burden in cancer genes suggests a greater contribution of germline alterations to the transformation process while late-onset cancers are more driven by somatic mutations.



nature COMMUNICATIONS

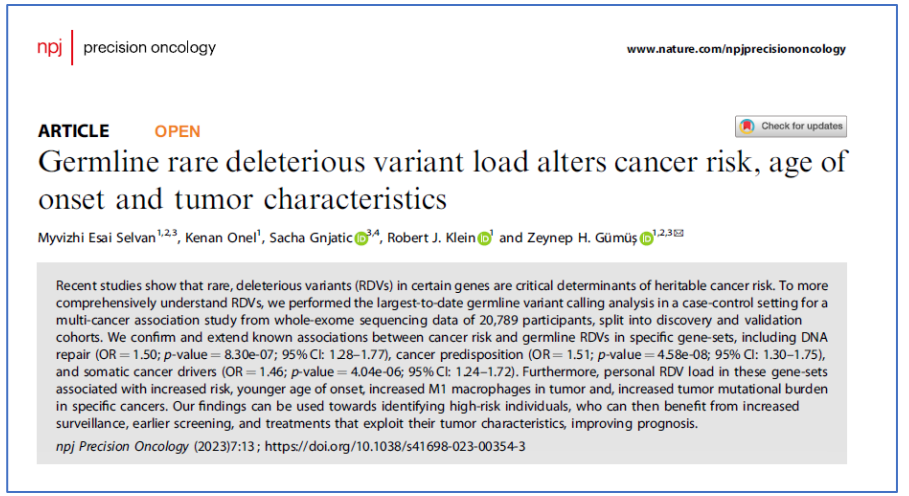
ARTICLE

https://doi.org/10.1038/s41467-022-31483-1 OPEN

The impact of rare germline variants on human somatic mutation processes

Mischan Vali-Pour[1,2], Solip Park[3], Jose Espinosa-Carrasco[4], Daniel Ortiz-Martínez[4], Ben Lehner[1,2,5] & Fran Supek[4,5]

Somatic mutations are an inevitable component of ageing and the most important cause of cancer. The rates and types of somatic mutation vary across individuals, but relatively few inherited influences on mutation processes are known. We perform a gene-based rare variant association study with diverse mutational processes, using human cancer genomes from over 11,000 individuals of European ancestry. By combining burden and variance tests, we identify 207 associations involving 15 somatic mutational phenotypes and 42 genes that replicated in an independent data set at a false discovery rate of 1%. We associate rare inherited deleterious variants in genes such as *MSH3*, *EXO1*, *SETD2*, and *MTOR* with two phenotypically different forms of DNA mismatch repair deficiency, and variants in genes such as *EXO1*, *PAXIP1*, *RIF1*, and *WRN* with deficiency in homologous recombination repair. In addition, we identify associations with other mutational processes, such as *APEX1* with APOBEC-signature mutagenesis. Many of the genes interact with each other and with known mutator genes within cellular sub-networks. Considered collectively, damaging variants in the identified genes are prevalent in the population. We suggest that rare germline variation in diverse genes commonly impacts mutational processes in somatic cells.



npj | precision oncology www.nature.com/npjprecisiononcology

ARTICLE OPEN

Germline rare deleterious variant load alters cancer risk, age of onset and tumor characteristics

Myvizhi Esai Selvan[1,2,3], Kenan Onel[1], Sacha Gnjatic[3,4], Robert J. Klein[1] and Zeynep H. Gümüş[1,2,3]

Recent studies show that rare, deleterious variants (RDVs) in certain genes are critical determinants of heritable cancer risk. To more comprehensively understand RDVs, we performed the largest-to-date germline variant calling analysis in a case-control setting for a multi-cancer association study from whole-exome sequencing data of 20,789 participants, split into discovery and validation cohorts. We confirm and extend known associations between cancer risk and germline RDVs in specific gene-sets, including DNA repair (OR = 1.50; *p*-value = 8.30e-07; 95% CI: 1.28–1.77), cancer predisposition (OR = 1.51; *p*-value = 4.58e-08; 95% CI: 1.30–1.75), and somatic cancer drivers (OR = 1.46; *p*-value = 4.04e-06; 95% CI: 1.24–1.72). Furthermore, personal RDV load in these gene-sets associated with increased risk, younger age of onset, increased M1 macrophages in tumor and, increased tumor mutational burden in specific cancers. Our findings can be used towards identifying high-risk individuals, who can then benefit from increased surveillance, earlier screening, and treatments that exploit their tumor characteristics, improving prognosis.

*npj Precision Oncology* (2023)7:13 ; https://doi.org/10.1038/s41698-023-00354-3

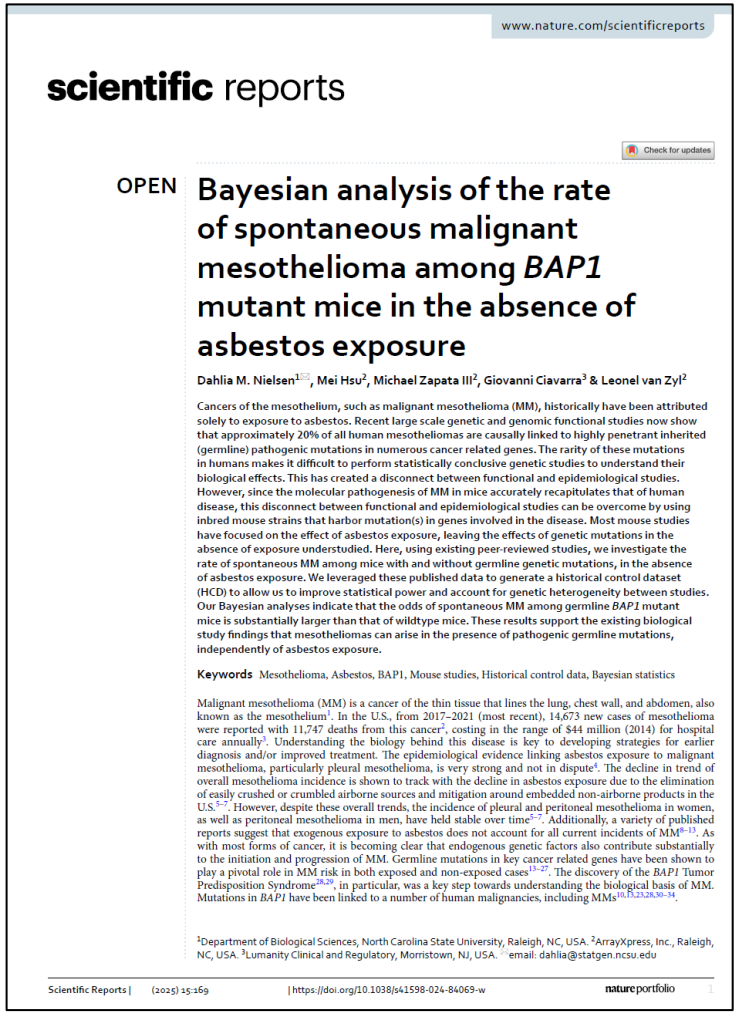
Qing *et al.*, 2020

Vali-Pour *et al.*, 2022

Esai Selvan *et al.*, 2023

# Highly Penetrant Pathogenic Mutations in the *BAP1* Gene Cause Spontaneous Mesothelioma and other Cancers Independent of Asbestos Exposure

*"Pathogenic mutations in BAP1 have been shown not only to initiate tumors, but to also drive the subsequent acquisition of endogenous mutations and epigenetic changes[85–90]. Much like TP53, studies have demonstrated that BAP1 functions within the parameters of all three classes of tumor suppressor genes: as gatekeeper (directly regulating cell cycle control and cell proliferation); as caretaker (regulating genomic stability); and as landscaper (regulating chromatin modification)[30,32,91,92]. By fulfilling the function of all three classes of tumor suppressors, is it not surprising that pathogenic heterozygous germline BAP1 null mutations can behave in a haploinsufficient manner, via either dominant-negative interactions or gain-of-neomorphic function(s) effects, ultimately causing cancer in nearly all germline carriers[28,31,32,93–96]."*



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## Bayesian analysis of the rate of spontaneous malignant mesothelioma among *BAP1* mutant mice in the absence of asbestos exposure

Dahlia M. Nielsen[1✉], Mei Hsu[2], Michael Zapata III[2], Giovanni Ciavarra[3] & Leonel van Zyl[2]

Cancers of the mesothelium, such as malignant mesothelioma (MM), historically have been attributed solely to exposure to asbestos. Recent large scale genetic and genomic functional studies now show that approximately 20% of all human mesotheliomas are causally linked to highly penetrant inherited (germline) pathogenic mutations in numerous cancer related genes. The rarity of these mutations in humans makes it difficult to perform statistically conclusive genetic studies to understand their biological effects. This has created a disconnect between functional and epidemiological studies. However, since the molecular pathogenesis of MM in mice accurately recapitulates that of human disease, this disconnect between functional and epidemiological studies can be overcome by using inbred mouse strains that harbor mutation(s) in genes involved in the disease. Most mouse studies have focused on the effect of asbestos exposure, leaving the effects of genetic mutations in the absence of exposure understudied. Here, using existing peer-reviewed studies, we investigate the rate of spontaneous MM among mice with and without germline genetic mutations, in the absence of asbestos exposure. We leveraged these published data to generate a historical control dataset (HCD) to allow us to improve statistical power and account for genetic heterogeneity between studies. Our Bayesian analyses indicate that the odds of spontaneous MM among germline *BAP1* mutant mice is substantially larger than that of wildtype mice. These results support the existing biological study findings that mesotheliomas can arise in the presence of pathogenic germline mutations, independently of asbestos exposure.

**Keywords** Mesothelioma, Asbestos, BAP1, Mouse studies, Historical control data, Bayesian statistics

Malignant mesothelioma (MM) is a cancer of the thin tissue that lines the lung, chest wall, and abdomen, also known as the mesothelium[1]. In the U.S., from 2017–2021 (most recent), 14,673 new cases of mesothelioma were reported with 11,747 deaths from this cancer[2], costing in the range of $44 million (2014) for hospital care annually[3]. Understanding the biology behind this disease is key to developing strategies for earlier diagnosis and/or improved treatment. The epidemiological evidence linking asbestos exposure to malignant mesothelioma, particularly pleural mesothelioma, is very strong and not in dispute[4]. The decline in trend of overall mesothelioma incidence is shown to track with the decline in asbestos exposure due to the elimination of easily crushed or crumbled airborne sources and mitigation around embedded non-airborne products in the U.S.[5–7]. However, despite these overall trends, the incidence of pleural and peritoneal mesothelioma in women, as well as peritoneal mesothelioma in men, have held stable over time[5–7]. Additionally, a variety of published reports suggest that exogenous exposure to asbestos does not account for all current incidents of MM[8–13]. As with most forms of cancer, it is becoming clear that endogenous genetic factors also contribute substantially to the initiation and progression of MM. Germline mutations in key cancer related genes have been shown to play a pivotal role in MM risk in both exposed and non-exposed cases[13–27]. The discovery of the *BAP1* Tumor Predisposition Syndrome[28,29], in particular, was a key step towards understanding the biological basis of MM. Mutations in *BAP1* have been linked to a number of human malignancies, including MMs[10,13,23,28,30–34].

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Nielsen *et al.*, 2025

# Approximately 20% of Mesotheliomas are Genetically-Caused

***"A minority of cases seem to be not clearly related to asbestos exposure and the incidence of these cases has been increasing in recent years, suggesting that genetic predisposing factors may play a crucial role (Gene Related-MPM). Moreover, familial cases of MPM have been largely evaluated in the recent past, suggesting that heredity may be an important and underestimated feature in MPM development. By analyzing the genetic susceptibility in MPM, approximately 20% of cases may be related to genetic predisposition: genes involved in DNA repair mechanisms are the most frequently involved."*** **Congedo *et al*., 2024**

# Gene-Related MMs vs Asbestos-Related MMs

**Table 3** Epidemiological and clinical differences between AR-MPM and GR-MPM

| Epidemiological and clinical differences | AR-MPM | GR-MPM |
|---|---|---|
| Association with asbestos exposure | Strong | Not strong |
| Age of presentation (years), (mean) | 72.3 | 56.3 |
| Gender ratio (M:F) | 5:1 | 1:1 |
| Symptoms frequency | +++ | +/− |
| Presence of pleural effusion | +++ | +/− |
| Stage at presentation | Usually advanced | Usually initial |

+++, common; +/−, rare. AR, asbestos-related; MPM, malignant pleural mesothelioma; GR, gene-related; M, male; F, female.

Adapted from Congedo *et al.*, 2024

# A Substantial Subset of Mesotheliomas are Genetically Caused

- A substantial subset (~20%) of mesotheliomas are genetically driven (*e.g.*, *BAP1*, *BARD1*, *BRCA1/2*, *TP53*, *NF2*, *etc*.) and constitute a distinct disease entity.
- **These tumors differ from asbestos-induced mesotheliomas across epidemiology, age at diagnosis, morphology, biology, prognosis, and management**.
- Key sources: Congedo 2024; Novelli 2024; Carbone 2025; Nielsen 2025, Wu 2025; Krevanko 2025

# *BAP1* Morphology & Pathology

- **Unique histologic patterns reported in *BAP1* carriers (multicompartment, less aggressive features).** (Carbone 2025; Wu 2025)
- Differential immunophenotype and molecular signatures compared to asbestos-related disease. (Carbone 2025; Wu 2025)
- **Implications for pathology workflows: integrate germline context when interpreting biopsies.**

# Diagnostic & Management Implications

- Screen for germline predisposition (*BAP1*, *BARD1*, *BRCA1/2*; *TP53*, and others) in young/low-exposure patients.
- **Adjust surveillance**: periodic imaging and multi-tissue assessment in *BAP1* carriers. (Carbone 2025; Wu 2025)
- **Therapeutic angles**: DNA repair vulnerabilities; epigenetic therapies; personalized trial enrollment. (Congedo 2024; Carbone 2025; Wu 2025)

# Similar Data for Numerous Other Highly Penetrant Genes

## Novelli *et al.*, 2024

PNAS RESEARCH ARTICLE | GENETICS

### Germline *BARD1* variants predispose to mesothelioma by impairing DNA repair and calcium signaling

Flavia Novelli[a], Yoshie Yoshikawa[b], Veronica Angela Maria Vitto[c], Lorenzo Modesti[c], Michael Minaai[a], Sandra Pastorino[a], Mitsuru Emi[a], Jin-Hee Kim[a], Franz Kricek[d], Fang Bai[e], José N. Onuchic[f], Angela Bononi[a], Joelle S. Suarez[a], Mika Tanji[a], Cristina Favaron[a], Alicia A. Zolondick[a,g], Ronghui Xu[a], Yasutaka Takanishi[a], Zhanwei Wang[a], Greg Sakamoto[a], Giovanni Gaudino[a], Joseph Grzymski[h], Federica Grosso[i], David S. Schrump[i], Harvey I. Pass[k], Lilit Atanesyan[l], Jan Smout[l], Suvi Savola[l], Kavita Y. Sarin[m], Hassan Abolhassani[n], Lennart Hammarström[n], Qiang Pan-Hammarström[n], Carlotta Giorgi[c], Paolo Pinton[c], Haining Yang[a], and Michele Carbone[a,1]

Affiliations are included on p. 11.

Edited by Peter Vogt, Scripps Research Institute Department of Molecular Medicine, La Jolla, CA; received March 13, 2024; accepted June 12, 2024

**We report that ~1.8% of all mesothelioma patients and 4.9% of those younger than 55, carry rare germline variants of the BRCA1 associated RING domain 1 (*BARD1*) gene that were predicted to be damaging by computational analyses. We conducted functional assays, essential for accurate interpretation of missense variants, in primary fibroblasts that we established in tissue culture from a patient carrying the heterozygous *BARD1*$^{V523A}$ mutation. We found that these cells had genomic instability, reduced DNA repair, and impaired apoptosis. Investigating the underlying signaling pathways, we found that BARD1 forms a trimeric protein complex with p53 and SERCA2 that regulates calcium signaling and apoptosis. We validated these findings in *BARD1*-silenced primary human mesothelial cells exposed to asbestos. Our study elucidated mechanisms of *BARD1* activity and revealed that heterozygous germline *BARD1* mutations favor the development of mesothelioma and increase the susceptibility to asbestos carcinogenesis. These mesotheliomas are significantly less aggressive compared to mesotheliomas in asbestos workers.**

genetics | carcinogenesis | mesothelioma | gene × environment | cancer prevention

**Significance**

There has been an unexplained increase of mesothelioma in younger patients who have not worked in the asbestos industry. We report that inherited germline mutations of *BARD1* cause some mesotheliomas in young patients. They experience significantly prolonged survival up to 20+ y and they require tailored screening and therapeutic approaches.

Cancer for the most part is a disease of old age, however, in recent years there has been an unexplained increase of cancer diagnoses among young patients. Various hypotheses, including exposure to increasing amounts of environmental carcinogens, have been proposed, yet there are no firm data to support these hypotheses (1). Mesothelioma, one of the best examples of a cancer caused by environmental carcinogens, is one of the malignancies that we see with increasing frequency in younger patients (2). This is very difficult to explain because asbestos causes cancer about 30 to 60+ y after initial exposure, thus most asbestos workers developed mesothelioma when they are old (2). Because, asbestos use was banned in the 80 s (2), former asbestos workers are now in their 70 s to 90 s, thus we should see mesothelioma in older not younger patients! (3)

In previous studies, we found that heterozygous germline mutations in the *BAP1* gene cause the BAP1 Cancer Syndrome, characterized by a high incidence of mesothelioma (4–9). We found that *BAP1*-linked mesotheliomas had a distinct clinical presentation: These patients very rarely had evidence of asbestos exposure, the median age of onset was 54 y old, several of them were in their 20 s and 30 s, the male to female and the pleural to peritoneal mesothelioma ratios were 1:1, compared to about 7:1 in mesotheliomas developing in asbestos workers (8, 10–12). Intriguingly, mesotheliomas developing in carriers of germline *BAP1* mutations had a median survival of 5 ~ 7 y and some were apparently cured as they survived mesothelioma for >20 y (8, 10–16). In contrast, mesotheliomas developing in asbestos workers have a median survival of ~1 y, are resistant to therapy, and are uniformly fatal (14). These differences point to different mechanisms underlying the pathogenesis of these malignancies. In additional targeted next-generation sequence studies we, and others, found that ~8 to 16% of mesotheliomas developed in carriers of germline *BAP1* mutations—the most frequent mutations—and, occasionally, in the contest of other tumor predisposition syndromes (8, 10–16). We also found some mesotheliomas developing in younger patients and associated with prolonged survival that did not contain mutations of any of the genes tested, which included those known to predispose to cancer (11). We suspected that additional genes, not included in our testing panel (11) might cause or predispose to less aggressive mesotheliomas in younger patients. It is important to identify carriers of germline mutations that predispose to cancer because screening of these individuals and of their affected family members for early cancer detection can be life-saving. Also, when diagnosed with cancer, these patients

Author contributions: F.N., V.A.M.V., L.M., J.-H.K., F.K., J.S.S., G.G., C.G., P.P., H.Y., and M.C. designed research; F.N., Y.Y., V.A.M.V., L.M., J.-H.K., F.K., A.B., J.S.S., C.F., A.A.Z., L.A., J.S., S.S., and H.A. performed research; Y.Y., M.M., S.P., M.E., F.K., F.B., J.N.O., M.T., R.X., Y.T., Z.W., G.S., J.G., F.G., D.S.S., H.I.P., L.A., J.S., S.S., K.Y.S., H.A., L.H., Q.P.-H., C.G., and P.P. contributed new reagents/analytic tools; F.N., Y.Y., V.A.M.V., L.M., M.M., S.P., M.E., J.-H.K., F.K., A.B., M.T., C.F., A.A.Z., Y.T., J.G., L.A., J.S., S.S., H.A., L.H., Q.P.-H., C.G., P.P., H.Y., and M.C. analyzed data; M.C. supervision; and F.N. and M.C. wrote the paper.

Competing interest statement: M.C. has a patent issued for "Methods for Diagnosing a Predisposition to Develop Cancer." M.C. and H.Y. have a patent issued for "Using Anti-HMGB1 Monoclonal Antibody or other HMGB1 Antibodies as a Novel Mesothelioma Therapeutic Strategy", and a patent issued for "HMGB1 As a Biomarker for Asbestos Exposure and Mesothelioma Early Detection." M.C. is a board-certified pathologist who provides consultation for pleural pathology, including medical-legal.

This article is a PNAS Direct Submission.



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Published July 11, 2024.



## Prassas *et al.*, 2025

*Cancer Medicine*

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RESEARCH ARTICLE OPEN ACCESS

### Estimating Cancer Penetrance in Carriers of *BRCA2* Pathogenic Variants Using Cancer-Specific Polygenic Scores

Brendan Prassas[1] | Zhuqing Shi[1] | Huy Tran[1] | Jun Wei[1] | Chi-Hsiung Wang[1] | Andrew S. Rifkin[1] | Annabelle Ashworth[1] | S. Lilly Zheng[1] | Ashley J. Mulford[2] | Alan R. Sanders[2,3] | Catherine E. Pesce[4] | Brian T. Helfand[1,3,4] | Henry M. Dunnenberger[5] | David Duggan[6] | Peter J. Hulick[5] | Allison DePersia[5] | Jianfeng Xu[1,3,4]

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**Received:** 8 April 2025 | **Revised:** 14 May 2025 | **Accepted:** 21 May 2025

**Keywords:** *BRCA2* | breast cancer (female and male) | hereditary breast and ovarian cancer syndrome (HBOC) | liver cancer | lung cancer | oral cancer | ovarian cancer | pancreatic cancer | polygenic score | prostate cancer

**ABSTRACT**

**Introduction:** *BRCA2* is a causal gene for hereditary breast and ovarian cancer (HBOC) syndrome. However, its association with other cancers and interplay with polygenic scores (PGS) remains unclear.

**Methods:** An observational cohort study for the diagnosis of various cancers in the UK Biobank (UKB, $N = 453{,}541$) were recruited at ages of 40–69 years Association of germline pathogenic variants (PVs) in *BRCA2* and published cancer-specific PGS with cancer risk was tested using Cox proportional hazards model.

**Results:** The median age and interquartile range (IQR) of participants at the analysis was 58.34 (50.60–63.74) years. Carriers of *BRCA2* PVs ($N = 1629$) had a significantly increased risk for four core HBOC-associated cancers (breast, ovarian, pancreatic, and prostate) and six additional types of cancer (lung, oral, small intestine, larynx, liver, and mesothelioma), hazard ratio (HR) > 2.37, all $ps < 0.001$. For eight cancers where cancer-specific PGS is available, each PGS was significantly associated with its respective cancer risk and independent of *BRCA2*, HR > 1.25 for 1 unit increase in standard deviation, all $ps < 0.001$. For female breast and prostate cancer, a significant interaction between *BRCA2* and PGS was found (HR < 0.83, $p < 0.05$); the effect of PGS on cancer risk was weaker in carriers than noncarriers. The probability of cancer by age 75 years ($P_{75}$) for these 10 cancers increased with higher PGS deciles in both carriers and noncarriers. For several cancers, the $P_{75}$ in carriers with the lowest PGS decile was lower than that of noncarriers with the highest PGS decile.

**Conclusions:** *BRCA2* PVs increase risk beyond core HBOC cancers and their risks are modified by cancer-specific PGS. These results suggest that genetic counseling of *BRCA2* PV carriers may extend to cancers beyond core HBOC syndrome and incorporate cancer-specific PGS in estimating their penetrance.

Brendan Prassas and Zhuqing Shi contributed equally to this study.

# Take-Home Conclusions

- **Inherited genetically-caused Malignant Mesotheliomas are Fundamentally Distinguishable from Asbestos-induced Malignant Mesotheliomas in Terms of Epidemiological, Clinicopathological and Prognostic Disease Features** (Congedo *et al*., 2024)
- **Compared to sporadic asbestos-induced MM, MM developing in germline *BAP*$^{+/-}$ mutation carriers-as well as in germline carriers of pathogenic mutations in other highly penetrant genes (*i.e.*, *BARD1*; *BRCA1/2*, *etc*.), are a different disease, biologically, histologically and clinically: these patients require a tailored diagnostic and clinical approach.**

# Take-Home Conclusions

- Large amounts of recent data allow experts in genetics to objectively and reproducibly distinguish between genetically-caused mesotheliomas and asbestos-induced mesotheliomas.
- Lawyers and experts need to truly understand the meaning of the recent data
- **Data shows that genetically-caused mesotheliomas differ from asbestos-induced mesotheliomas with respect to epidemiology, histopathology, molecular mechanisms, prognosis, and clinical management**.
- Recognizing this distinction changes screening, counseling, and treatment.

# *BAP1* Mutations Drive Mesothelioma Independent of Asbestos: Evidence from Genetically Engineered Mouse Models

# My Extensive Experience with Mouse Models

**Extensive Hands-on Experience:** PhD scientist, with 8+ years of laboratory experience designing and developing experiments using genetically engineered mouse models

**Range of Models:** Used a variety of mouse models to evaluate gene function in biological processes, including cancer

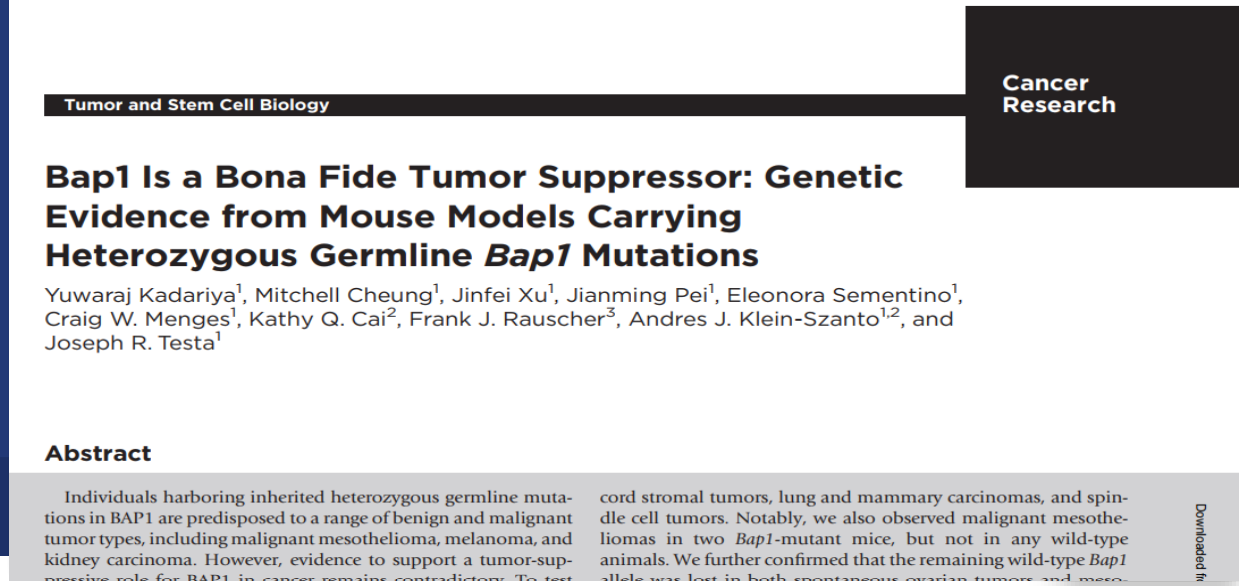
**Molecular and Phenotypic Analyses:** Skilled in conducting molecular and phenotypic analyses to validate genetic modifications and assess their biological effects

**Communication for Global Mouse Model Suppliers:** Design and implement communication programs for commercial lab animal vendors, helping convey the value and importance of mouse models to research

# Unlocking Mesothelioma Insights Using Mouse Models

- **BAP1 Mice:** Researchers have generated mice with BAP1 genetic mutations that mimic genetic changes found in some individuals who developed MM
- **Controlled Experimental Environment:** Genetically altered mice provide researchers with a powerful tool to study how MM develops in a very controlled experimental system (in the absence of asbestos)
- Littermates with and without the mutation (normal/wildtype) are raised using the same food, housing environment, climate, lighting, sleep patterns, etc.

# Unlocking Mesothelioma Insights Using Mouse Models (Continued)



Tumor and Stem Cell Biology

Cancer Research

**Bap1 Is a Bona Fide Tumor Suppressor: Genetic Evidence from Mouse Models Carrying Heterozygous Germline *Bap1* Mutations**

Yuwaraj Kadariya[1], Mitchell Cheung[1], Jinfei Xu[1], Jianming Pei[1], Eleonora Sementino[1], Craig W. Menges[1], Kathy Q. Cai[2], Frank J. Rauscher[3], Andres J. Klein-Szanto[1,2], and Joseph R. Testa[1]

**Abstract**

Individuals harboring inherited heterozygous germline mutations in BAP1 are predisposed to a range of benign and malignant tumor types, including malignant mesothelioma, melanoma, and kidney carcinoma. However, evidence to support a tumor-suppressive role for BAP1 in cancer remains contradictory. To test

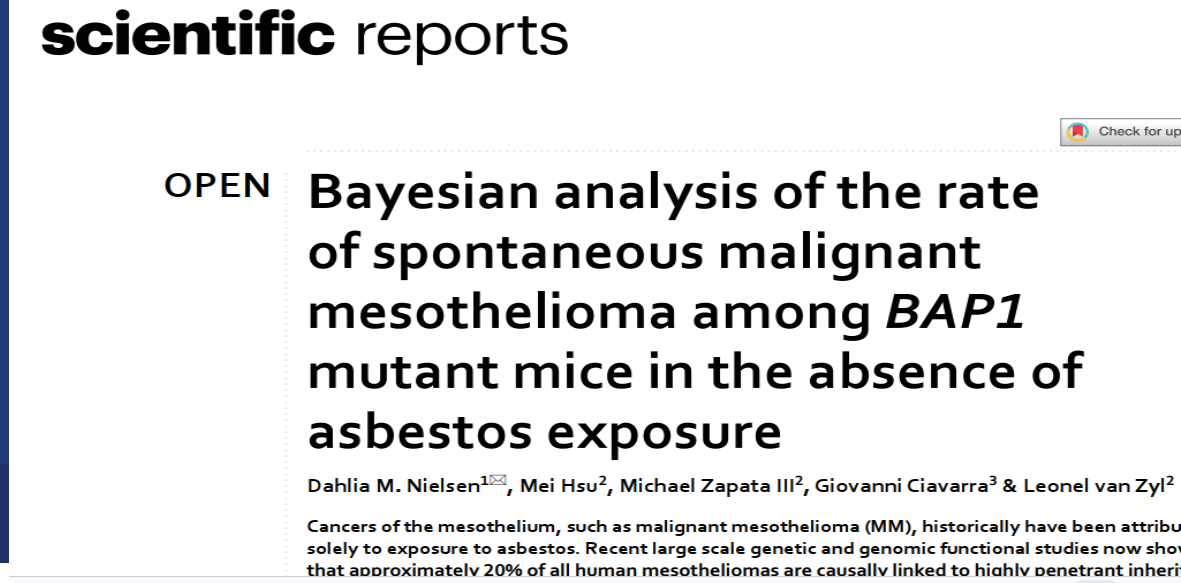
cord stromal tumors, lung and mammary carcinomas, and spindle cell tumors. Notably, we also observed malignant mesotheliomas in two *Bap1*-mutant mice, but not in any wild-type animals. We further confirmed that the remaining wild-type *Bap1* allele was lost in both spontaneous ovarian tumors and meso-



- **Beyond Asbestos Exposure:** Most mouse studies focused on effects of asbestos exposure; study by Kadariya et al. (2016) evaluated mice with both BAP1 mutations and no asbestos exposure
- Allowed assessment of whether BAP1 mutations alone could increase the risk of MM (independent of asbestos exposure)

- **2 out of 93 mice with BAP1 mutations developed MM spontaneously**, as compared to 0 of the 43 normal (wild type) control mice
- Results indicated that BAP1 mutations alone could cause MM, but the small number of mice coupled with the rarity of MM made it possible to deny significance using traditional statistical testing

# Well-accepted Statistical Approach Shows Stronger Evidence


scientific reports

Check for up

OPEN

**Bayesian analysis of the rate of spontaneous malignant mesothelioma among *BAP1* mutant mice in the absence of asbestos exposure**

Dahlia M. Nielsen[1✉], Mei Hsu[2], Michael Zapata III[2], Giovanni Ciavarra[3] & Leonel van Zyl[2]

Cancers of the mesothelium, such as malignant mesothelioma (MM), historically have been attribu
solely to exposure to asbestos. Recent large scale genetic and genomic functional studies now sho
that approximately 20% of all human mesotheliomas are causally linked to highly penetrant inheri

- We used a well-accepted statistical approach called Bayesian analysis to analyze Kadariya data since this approach is better suited for studying rare events like spontaneous MM (Nielsen et al., 2025)

- Using this method, we found:
  - 68.7% probability that mice with BAP1 mutations alone significantly increased MM risk – *this high probability merited further investigation*

- A 96.7-99.5% probability that mice with BAP1 mutations alone significantly increased the odds of developing MM without asbestos exposure when a historical control dataset was included in the analysis

- Per multiple other studies, additive and synergetic combinations of multiple pathogenic germline mutations may account for 20-36% of asbestos-independent genetic-related MM (Congedo, 2024; Belcaid, 2023)

***Our study provides new compelling statistical evidence that inherited genetic mutations in BAP1 can cause MM independent of asbestos exposure***

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# Increasing the Statistical Power of Animal Experiments by Using Data From Previous Studies (Historical Control Dataset)

- MM in normal wild-type animals is a rare event; need experiments with hundreds or thousands of animals (problematic from both a practical and ethical standpoint)
- Another method is to use a historical control dataset (HCD), which is a generally accepted method
- HCDs use pooled information on control animals from multiple, independent studies to characterize the natural incidence (background rate) of spontaneous tumors

***"Although the use of historical controls has been widely discussed in the literature, many researchers are unaware of their potential usefulness or do not know how to incorporate them into their experimental designs."*** **(Kramer, 2017)**

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# The Historical Control Dataset

- HCD built by investigating and compiling information on normal mice from existing published datasets; in total 8 MM in 8,627 mice

| Study | MM cases | Total # of animals | % MM |
|---|---|---|---|
| *Kadariya et al., 2016[37] | 0 | 43 | 0.00 |
| Mahler et al., 1996[67] | 0 | 243 | 0.00 |
| Radaelli et al., 2009[68] | 0 | 64 | 0.00 |
| Panchenko et al., 2016[69] | 0 | 69 | 0.00 |
| Huang et al., 2008[70] | 0 | 234 | 0.00 |
| Giknis and Clifford, 2005[71] | 6 | 6236 | 0.10 |
| Maita et al., 1988[72] | 2 | 1781 | 0.11 |
| Total | 8 | 8627 | 0.09 |

# Key Finding: *BAP1* Gene Mutation Alone Significantly Increases MM Risk

- **Combining historical control data with two different Bayesian statistical analyses:**

  ***Strong evidence was found that BAP1 gene mutations alone increase the risk of MM, without any asbestos exposure***

  ***Odds of a BAP1 mutant mouse developing MM were found to be 23 times higher than a normal (wildtype) mouse***

  ***Greater than 99.9% probability that BAP1 mutant mice have a higher rate of MM than normal (wildtype) mice***

  ***Strong evidence that BAP1 mutations can drive the development of MM, independent of asbestos***

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# Kadariya et al. (2025) Analysis

cancers

MDPI

Article

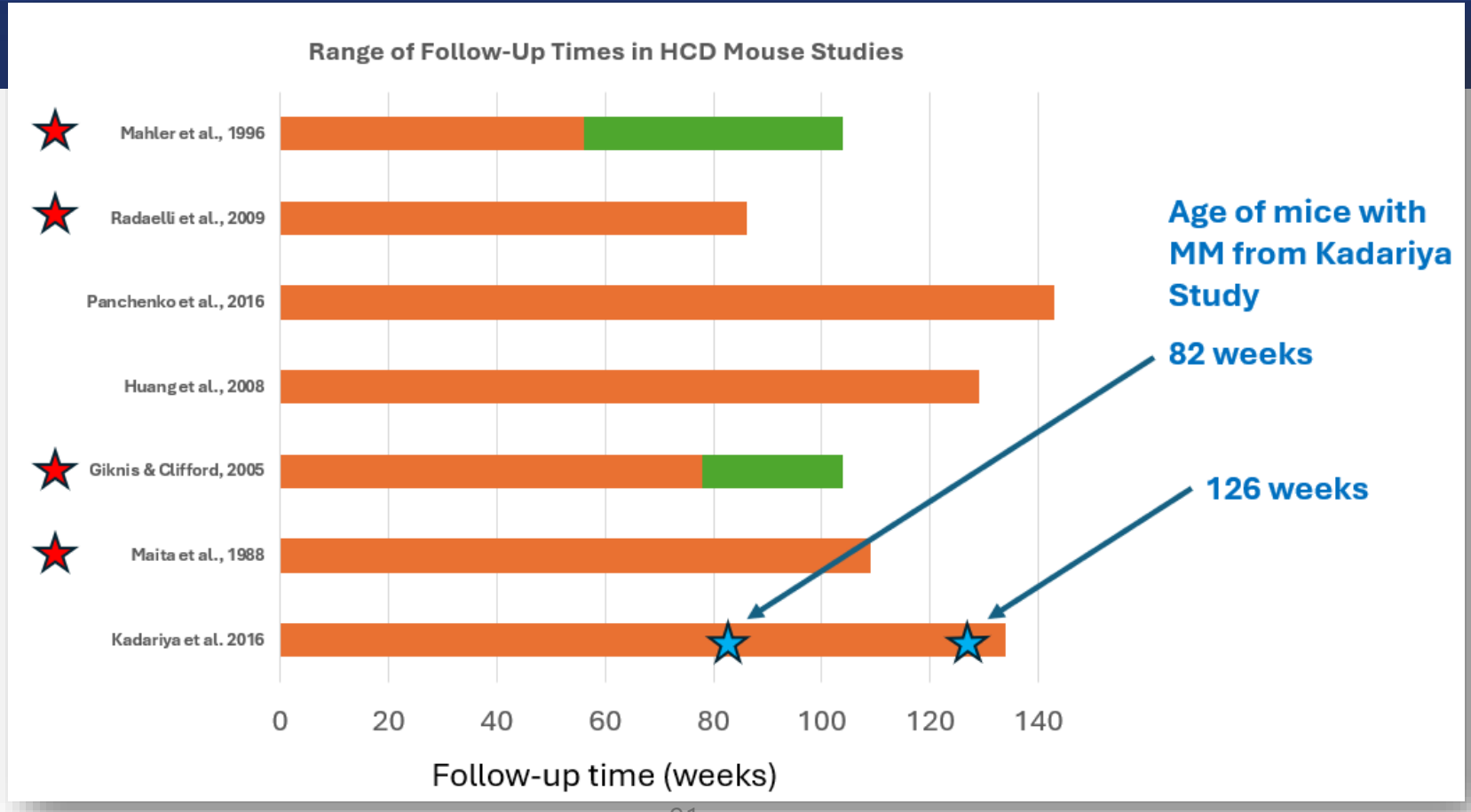
## Spontaneous Mesotheliomas in Germline *Bap1* Heterozygous Mice from Different Genetic Backgrounds

Yuwaraj Kadariya [1,†], Li Zhang [2,†], Eleonora Sementino [1], Eric Ross [2] and Joseph R. Testa [1,*]

- Authors suggested that Nielsen (2025) included studies that did not include MM that developed in the lifetime of mice
- However, four studies excluded in the Kadariya (2025) HCD covered mice through 82 weeks
- Thus, Kadariya (2025) was an underpowered study

# Kadariya (2025): Unduly Limited Controls

★ = Excluded by Kadariya (2025) in their analysis



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# Conclusions: Resolving a Key Scientific Question

- Previous statistical analyses of small mouse studies using t-statistics were used to deny that some germline BAP1 mutations were sufficient to cause MM on their own
- Using a more powerful and well-accepted statistical approach, Neilsen et al. demonstrated that pathogenic *BAP1* gene mutations are sufficient to cause MM on their own
- BAP1 mutations can drive the development of MM, independent of asbestos exposure

# Implications of Nielsen et al. (2025)

- The evidence provided by the Nielsen 2025 study further cements the need to use modern well-accepted statistical methods to evaluate genetic factors as the cause of MM in a significant number of legal cases, especially when exposure to asbestos is questionable

- This perspective could provide pivotal evidence in litigation, potentially leading to different legal outcomes based on the genetic predispositions of plaintiffs

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# More Information Than You Think!

## aka – don't fall into traps

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# Acquiring and Utilizing Genomic Data

- More information than you think
- Genetic Data Glossary
- Medical Records and Other Sources of Data
- Clinical Providers (No relevant clinical data has been found)
- Underlying Data (VAF Importance)
- Annotation of the Data
- Next Generation Whole Genome Sequencing (Long Read, Short Read, RNAseq)
- Flexible Reporting and Panel Development
- The Traps

# More Information Than You Think

- Bottom line – what you see in the medical records is the tip of the iceberg and this information is useful for both plaintiffs and defendants
- Several reasons for limited information being provided to the clinicians
- The Whole Genome Sequence contains all the information about the genome, including tens of thousand of variants and deletions
    - Exome sequencing only covers a very small portion of the genome in the coding regions
        - Panels make up a small portion of the variants in the exome sequencing
            - The clinical report makes up a very small portion of the data reported from the “panels”

# Refresher on Genetic Data Terms

- Germline vs. Somatic
- Exome vs. Whole Genome
- DNA vs RNA
- Clinical Report vs. Data
- Varying File Formats
- Varying Information Within the Annotated Files
  - VAF %
  - ClinVar
  - CADD
  - Location
  - Type of mutation (SNV, Deletions, Insertions, and Copy Number Variations (CNV))

# Existing Genetic Reporting

- With sequencing becoming the gold standard for diagnostics and treatment decisions, very often, if clinical selection criteria are met (think screening), some type of genetic testing has been done
- WGS is the preferred testing method both for the information available and the economic benefits, however
- USUALLY, the reports in the medical records only have a limited amount of data
- This data, while very limited, can be useful, but you have to dig to find the real information
- **The commercial sequencing business has the data with no new sample collection required**
- This is not data that document collection services understand, know what to look for, or can handle
- Be specific in your records requests

# Sources of the Genetic Results

- You find the genetic information in medical records, depositions (if you are a defendant), and genealogy – decide if it is a hereditary genetic case or not
- Reports may readily exist within the discovery information from clinical providers (Invitae, Tempus, etc), the hospital systems, core labs at university research hospitals, and research studies that often have “off book” testing
- **Do not take “No relevant clinical data has been found” to mean “no mutations”**
- Let the selection criteria and the data guide you and migrate from a quantitative to a qualitative understanding

# Getting the Underlying Data (Formats and Access)

- The data itself can be raw sequencing files, output files, spreadsheets, or other proprietary formats
- For whole genome sequencing the raw data can be in the terabytes and take many hours to download for processing, but it is just raw data
- Exome data is in the gigabytes because it is a fraction of the gene regions
- Some commercial vendors now have a portal where you establish an account and are granted access - other vendors will send you an FTP site
- Legacy vendors sometimes want to ship you the data on a hard drive
- **Focus on the data**, not the provider

# Making Value From the Data

- Get or Have the Data Annotated
- Data That Can Be Useful
    - VAF % is an example of the information that is usually found but not always reported
    - Rarity (population frequency)
    - Specific locations on the variants for databases and predictive scoring (CADD)
    - VUSs are often not reported but are in the data and can work in combinations
    - Penetrance, microsatellite instability, tumor burden, etc. is often discoverable
- RNAseq is now mainstream (expression levels and the transcriptome)

# New Sequencing (Short Read, Long Read, RNAseq)

- **Planning**
  - PLAN AHEAD – this takes time
  - First you must get the court issues resolved – including the breadth of the sequencing (full or ”panel”)
  - Pick a sequencing facility or a partner with access to one that meets the requirements – WGS, NOT EXOME
  - Placing the order, both for the draw and the sequencing
  - Tissue collection and shipment occurs (blood, margin tissue, hair follicles, skin punch biopsy, salvia if you must)
  - Implementation of controls and processes with the sequencing facility – they are unaccustomed to restrictions
- **Receiving the Data**
  - Wait – with rush fees it can be as fast as two weeks to data production, realistically, a month
  - The facility will make everything available to plaintiff and defense experts simultaneously (exception is QC)
  - Raw data can be downloaded from an FTP site – can be in the terabytes
  - If no special filtering or bioinformatics are requested, direct costs are in the $3,500 range (per individual)
- **Bioinformatics**
  - Can be completed in layers since all the data exists
  - Focus on relevant pathways known to impact the disease onset and progression
  - Start with the obvious pathogenic and likely pathogenic (updated daily) and VUS’ with CADD of 20 or greater
- **Assessment**
  - Compare to the published peer reviewed literature (large volume filtering) – new publications weekly
  - Determine mode of action and pathways and report

# Flexible Reporting

- DNA (and RNA) are sensitive topics – let your client or if you are the defendant, your experts, focus only on the disease at hand
- You cannot predict which specific genes, we have seen that over and over
- The results are somethings very different than the conclusions drawn by treaters with limited information, and can have profound effects on outcomes
- As a scientific team we want to know all the relevant facts, but sometimes the legal environment restricts that
- Privacy is managed by limiting the scope of the data reported by the experts, and if needed, reported to the expert(s)
- You can follow a process of building a disease specific panel from the literature and then if needed, expand beyond that

# The Traps

- If you are a plaintiff, don’t assume that genomics is something that is going to work against you, knowledge is power
- If you are a defendant, don’t assume all cases can be explained with hereditary genetics
- Don’t fall into the biggest trap of assuming a no clinical findings report means there are no pathogenic variants driving the disease
- Please don’t blindly restrict the sequencing data from becoming part of the medical research body of knowledge. Ultimately, we ALL want to find cures for cancers

# Expanding Case Applications

- Rare diseases – majority of the projects we have historically seen
- Broadening to more common diseases with a wider array of possible toxicants
- Birth defect cases with an exposure to a parent are increasing

  Trio sequencing for birth defects and parental exposure cases

  - *de Novo* mutations diagnosed but mutation not present in parents
  - Can determine from a parent's fragment if the DNA came from them

- Exposure signatures – somatic for radiation, benzene (active), EtO (active), smoking, etc. and ASBESTOS is coming

# ToxicoGenomica

Multidisciplinary group of geneticists, scientific consultants, and counsel offering consulting and expert services in genomics & systems biology regarding toxins



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