

# Your Presenters



Complimentary CLE Webinar

## Updates on BAP1 and BRCA Genes

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# Webinar Overview

- First, I will comment on law and science developments
- Second, Dr. Len van Zyl will address key recent scientific studies, including:
  - his team's Nielsen 2025 study regarding BAP1
  - large scale studies further establishing that germline mutations cause many cancers
  - studies explaining the mechanisms involved in cancers caused by BRCA1 and 2
- Third, Michael Zapata will focus on acquiring and utilizing genomic data via, including:
  - genetic data in medical records
  - subpoenas to sequencing operations
    - commercial sequencing companies such as Invitae, Tempus, etc
    - hospitals with internal sequencing services

# Law and Science Update

- Recent Developments
- Some Key Points/Reminders from Prior Webinars
  - Whole Genome Sequencing (WGS)
  - Stipulations and Orders for Extensive, Flexible Sequencing
  - Flexible Reporting of Sequencing Results Can Eliminate Inefficiencies and Disputes

## New Roggli et al Article:

**“Recent trends in the causation of peritoneal mesothelioma: fiber burden analysis of ten cases”**

- Roggli VL, Novakovic S, Ghio AJ, Li H, Pina-Oviedo S, Carney JM, Sporn TA, Glass CH, Pavlisko EN. Recent trends in the causation of peritoneal mesothelioma: fiber burden analysis of ten cases. Ultrastruct Pathol. 2025 Mar 26:1-8.
  - <https://pubmed.ncbi.nlm.nih.gov/40143456/>

“We performed fiber burden analysis on lung tissue in 10 cases (six men, four women) of peritoneal mesothelioma since 2010.”



## More re New Roggli et al Article

- Key quotes from abstract:
- “The median age for the six men was 62 years (range: 53-75 years). Three cases were epithelioid type and three were biphasic. Two of six cases (33%) had an elevated lung fiber burden, with one case exclusively crocidolite and the other predominately amosite.
- The median age for the four women was 55 years (range: 39-63 years). Two cases were epithelioid type and two were biphasic. None of the four had an elevated lung fiber burden.
- Our findings are consistent with contemporary epidemiological studies indicating that a minority of peritoneal mesotheliomas occurring in men are asbestos related and very few are asbestos related in women.”

# **Mizer Update – WGS and Flexible Reporting Used**

## Reminder re Specifics of Mizer & Stipulation to WGS and Flexible Results Reporting

- Mizer is an ongoing New Jersey case; spring and summer 2024 orders from Judge Viscomi essentially Mizer up as a test case for NJ regarding genetic testing in asbestos cases
- Moving defendants filed an extensive brief and supporting declarations on July 30, 2024
  - Declarations by Len van Zyl, Ph.D, Laura Li, Ph.D. of BT Genomics, and Dr. Allan Feingold
- Instead of filing a response brief or evidence, plaintiff's counsel (Maune Raichle) stipulated to WGS and initial reporting of results for over 400 genes
- Initial results to be reported include all 81 genes listed in Tables 2 and 3 in Belcaid 2023, a review of reviews listing genes known to have been found to carry germline mutations in people with mesothelioma
- Judge Viscomi so ordered the stipulation but noted:
  - *The court has approved the stipulation reached by the parties, but this should not be interpreted as the court taking any position on the issue being litigated.*

## WGS and Flexible Sequencing Utilized

- During the winter, blood draw and WGS took place
- Flexible DNA sequencing results reporting took place
  - results are not public
- Dr. van Zyl timely filed his report in January 2025
- Plaintiff did not file a report by deadline and did not file a motion to extend time
- Current schedule calls for:
  - expert depositions to be done by late June
  - Daubert hearings completed in September
  - October 6 - trial starts

# Notable Recent Genetic Testing Ruling

# March 10, 2025 Genetic Testing Ruling - Combs – Alameda

- Attention should be given to recent proceedings before Judge Patrick McKinney in the Combs case in Alameda County; *23CV056760: COMBS, et al. vs ABB INC., et al.*
- Contra Costa and other defendants moved for sequencing of 28 genes, relying on a declaration by Dr. Lewis A. Chodosh and the genes listed in a mesothelioma-specific gene panel offered by the University of Chicago
- Plaintiff opposed sequencing of genes other than *BAP1*; plaintiff relied on a declaration by Dr. Wayne W. Grody
- The experts were **not** deposed.

## More re Genetic Testing Ruling - Combs

- Judge McKinney heard oral argument after issuing a 2/5/25 tentative order limiting sequencing to BAP1; the argument transcript deserves review
- Ultimately, Judge McKinney issued a 2/10/25 order which mainly retained the tentative order, but at page 2 also included language allowing additional expert testimony:

“At the hearing, all counsel seemed to understand that this was cutting edge medical research; and that evidence of a plausible causal link between a germline mutation of BARD1, for instance, and mesothelioma may be discovered in the coming years. Recognizing that these developments might happen while this case is pending, the Court permits Defendants to renew their motion to compel genetic testing of germline mutation of one of the identified genes if and only if a qualified individual can testify that additional cancer research has demonstrated a plausible causal link between the identified germline mutation and mesothelioma unrelated to exposure to asbestos.”

# More re Genetic Testing Ruling - Combs

- Contra Costa and plaintiff did **not** submit additional expert testimony
- In many ways, the declarations were 2 ships passing in the night as they addressed different aspects of genomics
- Both declarations did not mention Dr. Testa's 2022 concessions in Watts that 1) genetic abnormalities can cause mesotheliomas, and 2) mutations in a particular person are unpredictable
- Both declarations also did not cover key recent studies on genomic causation, such as:
  - 1) Congedo 2024 (estimating genetic causation of 20% of mesotheliomas)
  - 2) Febres-Aldana 2024 (MSK researchers acknowledge genetic causes of mesothelioma)
  - 3) Nielsen 2025 (Dr. van Zyl's 2025 study showing over 90% Bayesian certainty that a single null variant BAP1 mutation can initiate and drive mesotheliomas and other cancers)



## More re Genetic Testing Ruling - Combs

- Contra Costa took a writ seeking interlocutory review; the submission was comprehensive, including the declarations and transcript of oral argument; see First District docket # A172581
  - [https://appellatecases.courtinfo.ca.gov/search/case/disposition.cfm?dist=1&doc\\_id=3124683&doc\\_no=A172581&request\\_token=OClwLSEnTkw8WzBRSCI9WEtIUFW6UVxfJCI%2BUzJTLdtOCg%3D%3D](https://appellatecases.courtinfo.ca.gov/search/case/disposition.cfm?dist=1&doc_id=3124683&doc_no=A172581&request_token=OClwLSEnTkw8WzBRSCI9WEtIUFW6UVxfJCI%2BUzJTLdtOCg%3D%3D)
- Intermediate appellate court summarily denied the writ request; Contra Costa has a pending request for review by the California Supreme Court – see docket S289786
- Writ papers include hearing transcript, expert declarations, and motion papers
  - <https://www.dropbox.com/scl/fo/btg9ywa79hb3ova1zx880/ALYFlqAFBPBYkkB39lhWa1M?rlkey=ji0rdtlp8750in82sjsd997rs&e=1&dl=0>

# Flexible Sequencing

## Flexible Reporting of Sequencing Results Reduces 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

# Genomic Evidence

# Genomic Evidence is Admissible and Can Be Dispositive

- Contrary to the claims of some lawyers, genetic evidence has been admitted in court on disease causation
- In fact, some courts have entered summary judgment orders for defendants based on the strength of genetic evidence
- Those orders include the seminal 2006 case in which summary judgment was entered for the defense after genetic testing established 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)
- Another example is *Ortega v. United States*, 2021 U.S. Dist. LEXIS 188969, 2021 WL 4477896 (“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.”)

## Granillo - Plaintiff's MIL re ALK Gene Fusion

- Granillo case is set for trial this spring, and involves a mesothelioma in a woman under 30; # 22 STCV 14155 – Los Angeles
- In general, several experts retained by defendants opined mesothelioma was caused by an *ALK* fusion which arose from genomic instability induced by *BRCA1* mutation, citing 21 case reports
- Maune firm MIL #4 argues that defense expert opinions should all be excluded as speculation because there are no epidemiologic studies of women with pathogenic *BRCA1* mutations, *ALK* fusions and mesothelioma

## Granillo - Plaintiff's MIL re ALK Gene Fusion

- Per Maune Raichle motion, 21 case reports are not enough:
  - “B. The Proffered Opinions, Based Solely on Case Reports of Approximately 21 Individuals World-Wide is Marginally Probative but Will Greatly Confuse the Jury and Ev. C. 352 Requires Exclusion”
- Defendants could be arguing that plaintiff firms that oppose genetic testing are in essence involved in spoliation by destroying/blocking the gathering of evidence
- Citing Maune Raichle's MIL, defendants also could be arguing that plaintiff courts are depriving defendants of due process if they 1) block whole genome sequencing and 2) block experts from re-using genetic data in multiple cases

## Plaintiff Tactic – Ignore Data and Focus on Words Not In the Article

- Scientific studies are based on data
  - “In God we trust, all others must bring data”
    - Generally attributed to W. Edwards Demming
    - <https://www.ibm.com/think/insights/in-god-we-trust-all-others-must-bring-data#:~:text=%E2%80%9DIn%20God%20we%20trust.,data%20analysis%20is%20equally%20important>
- Regarding genomic findings, plaintiff tactics often appear to focus on ignoring data and instead focusing on words not in an article



# Challenges to Differential Etiology

- Granillo plaintiff MIL#4 and defense motions illustrate challenges to etiology opinions
- Plaintiff's can challenge opinions of defendant retained experts who state conclusions but are not expert in genetics
- Defendants can challenge the scientific reliability of an etiology opinion rendered by a witness (e.g. Drs. Kradin and Zhang) who automatically attributes causation to a known or unknown exposure to a toxicant such as asbestos
- For genetics in particular, experts may be excludable due to:
  - failure to actually analyze recent scientific literature
  - failure to actually analyze the cancer syndrome significance of a personal or familial history of cancers or other non-cancer diseases/conditions
  - failure to utilize reliable, reproducible methods and data, including calculation of CADD scores, assessment of Hallmarks of cancer, and assessment of VAF %s

## Dr. Kradin Excluded for Failure to Address Alternative Causes

- Keller v. Exxonmobil Oil Corp., 2025 U.S. Dist. LEXIS 44043
  - <https://asbestoscasetracker.com/summary-judgment/defendants-motionfor-summary-judgment-granted-based-on-expert-preclusion/Text>
- “Dr. Kradin's subsequent deposition testimony regarding his skepticism about radon (Kradin Tr. at 26-27) may have merit, but this post hoc rationale, which is not in any way memorialized in his report, does not indicate that the methods he previously employed to form the opinions contained in the report were reliable. (Id. at 25 (“A. I didn't look at any other contributing factors [other than asbestos and secondhand smoke]. Q. Were you asked to look at radon? A. I wasn't asked to look at radon.”)); see also, e.g., Wills, 379 F.3d at 45-46 (finding that expert's failure to account [\*24] for smoking and alcohol habits in reaching conclusions regarding cancer causation, despite concession that they were major risk factors, indicated that the conclusions were “not grounded in reliable scientific methods”).

# Common Genomic Mistakes to Avoid

- Classification of a mutation as a VUS (variant of unknown significance)
  - is not gospel; classifications change
    - Waters 2024 changed classification for many for *BAP1* mutations
  - does not mean the mutation is benign
- “Paper” sequencing reports often exclude huge amounts of data; obtain digital data
- Reports about “actionable” mutations are not complete
- Two benign mutations can be lethal if located “in cis”

## “Benign” Variants May Not Be Benign – *BARD1* example

- Novelli *et al.* 2024: rare heterozygous pathogenic germline variants in the (*BARD1*) gene cause malignant mesothelioma, particularly in young patients with no known history of asbestos exposure
- Li et al (2021) reported the discovery of two *BARD1* mutations, namely p.Pro24Ser and p.Arg378Ser, simultaneously in patients of a hereditary breast and ovarian cancer family.
- *“These BARD1 mutations, in isolation, cause no readily obvious phenotype; but when combined in cis, they dramatically impaired the DNA Damage Response (DDR) pathways leading to rapid genome wide instability in affected cells, resulting in tumors in vitro and in vivo.”*
- *Cis* effects are caused by genetic variants located on the same DNA molecule
- Neither of the single mutations causes a functional change, but together they synergistically impair the DNA damage response and lead to tumors *in vitro* and *in vivo*.

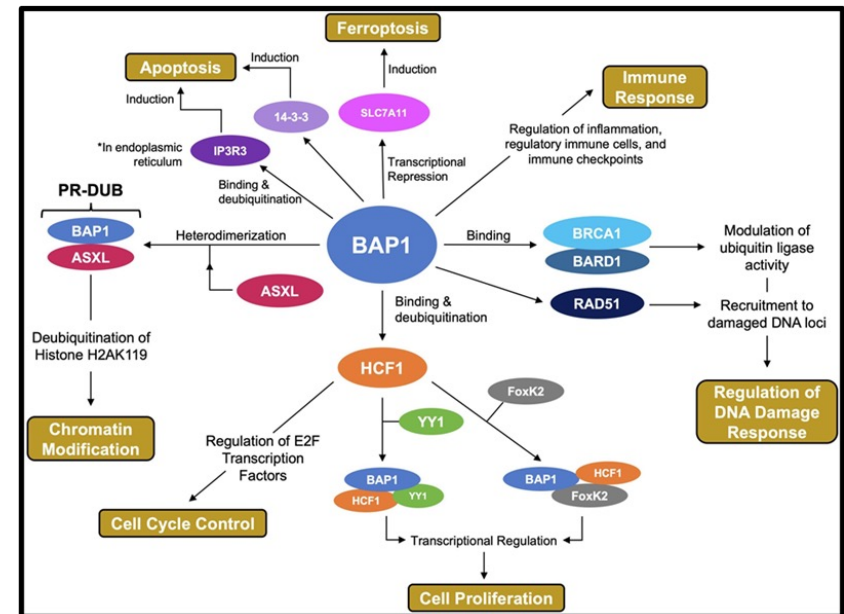
# BAP1

# BAP1 Update and Limits of Sequencing Methods

- All stakeholders need to understand Nielsen 2025, the recent study by Dr. van Zyl and colleagues
- In Cowger, Combs and other cases, plaintiffs explicitly or implicitly conceded to DNA sequencing of BAP1
- Stakeholders need to understand that sequencing methods are **not** fungible; WGS provides the most scientific certainty
  - exome sequencing of BAP1 is **not** dispositive of whether a mutation is present because exome sequencing omits about 98% of each gene and has other flaws

# Proteins Interact – One Mutation Can Impair the Function of Many Proteins

- BAP1 stands for **BRCA1-Associated Protein 1 (BAP1)**
- BAP1 protein interacts with many other proteins
- TP53 protein interacts with even more proteins



Adapted from Louie and Kurzrock, 2020

# BRCA1 and 2, BARD1 and Others



# Recent Evidence on Germline Mutations in Non-Smokers, Including Germline BRCA1 and 2

- Germline mutations of BRCA1 and 2 are pertinent to more than breast cancer and ovarian
- Germline mutations of BRCA1 and 2 also can cause lung cancers, mesotheliomas, prostate cancers, pancreatic cancers and
  - Carapezza G, Minardi SP, Noci S, et al. Germline Whole-Exome Sequencing in Non-Smoker Lung Cancer Patients Reveals Pathogenic Variants in Lung Cancer Driver Genes. *Genes Chromosomes Cancer*. 2025;64(3):e70040.

## ABSTRACT

- “Approximately 10%–15% of all lung cancers arise in non-smokers.”
- “Seven of the genes we identified (54%) are involved in DNA repair (ATM, BRCA1, BRCA2, FH, MLH1, MUTYH, and TP53) [26] suggesting that variants in this pathway may cause deregulation of DNA repair and cell cycle and increase cancer risk.”
- “Whole-exome sequencing was performed on genomic DNA from peripheral blood Overall, our results strongly support the concept that non-smokers lung cancer patients carry PGVs [pathogenic germline variants] in cancer predisposing genes, especially in lung cancer driver genes, and are directly and/or indirectly involved in cancer development.”

## Some BRCA1 and 2 Germline Mutations Make Smoking and Drinking Relevant to Mesos

- Contrary to conventional wisdom, smoking has always been relevant to mesothelioma, as previously pointed out by Dr. Moolgavakar in Dr. Testa's book on mesothelioma; epidemiologists missed the effects for various reasons
- Dr. van Zyl's slides quote studies that explain that aldehydes cause endogenous genomic abnormalities
- Aldehydes arise from many sources, including smoking and drinking

## More re Aldehydes and Cancers

- Aldehydes can have even stronger effects in people with pathogenic *BRCA1* and 2 mutations and Type 2 diabetics by degrading protein from a normal copy of a gene
- Same mechanisms at work for cancers attached to many organs
- Same mechanisms may well be at work with mutations of other genes

# Learn Endogenous Mechanisms

- Dr. van Zyl's slides show multiple endogenous (internal) mechanisms that cause cancers
- Stakeholders need to really understand these mechanisms because they explain why some cancers arise independent of external exposures

## "2 Hit" Theory Has Many Exceptions

- Dr. van Zyl's slides show an example of endogenous mechanisms can cause exceptions to "2 hit theory"
- Stakeholders need to really understand the exceptions to 2 hit theory
- "Tissue specific" haploinsufficiency is an important concept
- Many TSG's can act in a haploinsufficient manner for tissue specific reasons

## Dr. Moline Ordered to Turn Over Names of People Who Were Utilized in Studies

- Prior experiences have shown that Dr. Moline's etiologies often fail to meaningfully address personal and family histories of cancers and other diseases
- Per a recent order by Judge Silvera (key quote below), April 8 is the date for Dr. Moline to turn over the records identifying the people who were the subjects of her "studies."
  - Johnson & Johnson v. Northwell Health Inc, 2025 N.Y. Misc. LEXIS 1839
    - "Nonetheless, in light of the Court of Appeals' decision denying Northwell's motion for leave to reargue its motion for leave to appeal, see NYSCEF Doc. No. 125, this Court orders Northwell and Dr. Moline to comply with the First Department's Decision by turning over the documents requested in the Subpoenas by April 8, 2025."
- Stakeholders will need to investigate and understand cancer and other histories from subjects of studies

# Reminders

# Genetic Testing Results Are Often in Medical Records



## 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

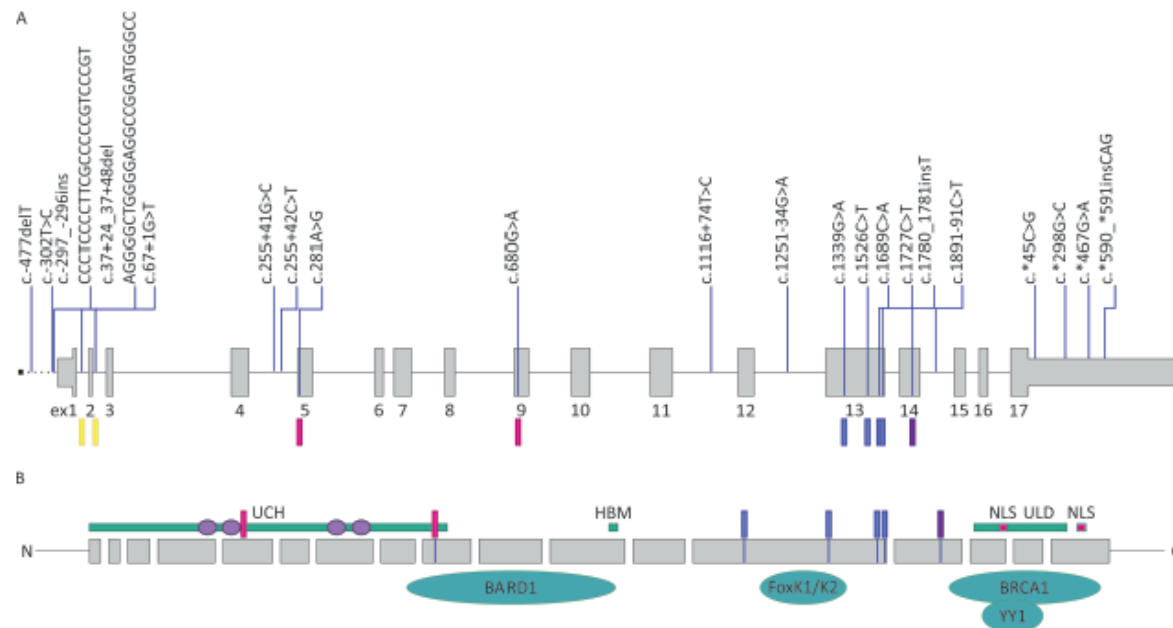
# Whole Genome Sequencing Usually is Key

# 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 not 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.)

# Pathogenic Mutations Exist in Intronic and Other “Regulatory” Regions

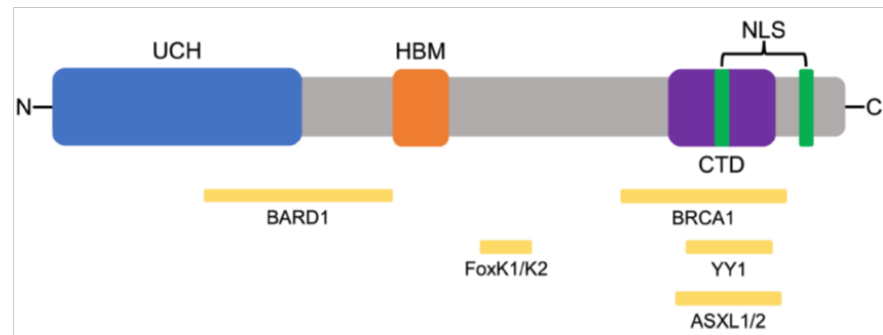
- Similarly, multiple peer reviewed published studies have also highlighted the importance of screening deep intronic regions of *BAP1* gene for pathogenic mutations (i.e., Repo *et al.*, 2019; Ohar *et al.*, 2016 etc.)



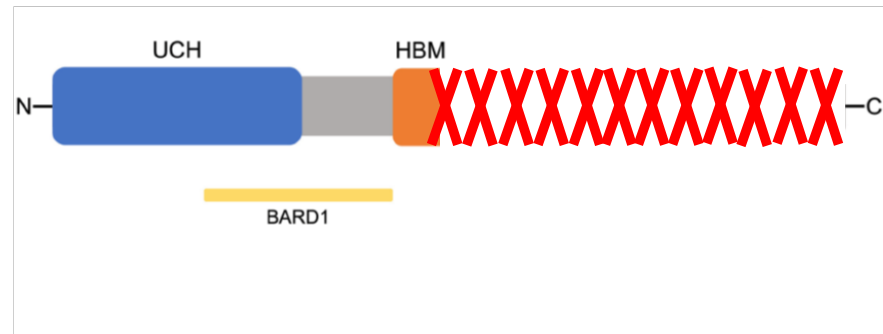
Adapted from Repo *et al.*, 2019

## Specifics Matter : Pathogenic *BAP1* p.Arg385Ter - A Truncating Null (Loss-of-Function) Germline Mutation

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/>

ToxicoGenomica

# **“Genetic Defenses” are Not All the Same**

## “Genetic Defenses” are Not All the Same

- Some stakeholders **incorrectly** argue as if all “genetic causation defenses” are the same
- In fact, the extent and power of a genetic causation defense depends on multiple factors
- Generally speaking, “genetic causation defenses” can and do fall into multiple different “buckets” with different sets of supporting evidence

# “Replication Error” Defense

- For example, a “replication error” genetic defense has relatively little factual focus on the particular plaintiff
- A “replication error” defense is based mainly on the general principles that 1) pathogenic mutations can and do occur when cells divide themselves (mitosis) in order to produce new cells (replication), and 2) 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



# Defense Based on Plaintiff Specific Mutations

- In contrast, other “genetic causation defenses” are based on the results of genetic sequencing results specific to the plaintiff, often including personal and familial medical histories of cancers and/or other conditions
  - Key aspects of this defense are based on results of germline sequencing and/or somatic sequencing (tumor sequencing)
  - Additional aspects involve application of reliable, reproducible methods and date, including calculation and presentation of CADD scores and VAF %s
- To the best of my knowledge, no one has tried such a case to verdict
- I can say with certainty that no one has tried to verdict a case in which Dr. Len van Zyl has been called to testify

# CADD Scores

## CADD Scores Can Play a Significant Role in Differentiation Between Disease Causing and Benign Gene Variants

- CADD analysis provides an objective, quantitative method of assessing likely pathogenicity of gene variants (mutations) (Kircher *et al.*, 2014; Rentzsch *et al.*, 2019; 2021).
- CADD scores are especially useful when functional empirical evidence is lacking or insufficient to make an evidence-based determination.
- CADD integrates over 60 existing commonly used predictive algorithm tools, each with their own criteria and inherent biases (Kircher *et al.*, 2014; Rentzsch *et al.*, 2019; 2021).
- The resulting CADD score provides an objective, quantitative metric of deleteriousness/pathogenicity to better guide the treatment options and research directions available to clinicians and researchers (Kircher *et al.*, 2014; Rentzsch *et al.*, 2019; 2021).

## General Meaning of CADD Scores

- **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).

# CADD Scores for Gene Variants in Mesothelioma Research Studies (Dr. Testa - Cheung *et al.*, 2021)

Human Molecular Genetics, 2021, Vol. 30, No. 18 | 1753

**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:c.7024_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

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Len van Zyl, Ph.D.  
10 April 2025



# Overall Conclusions

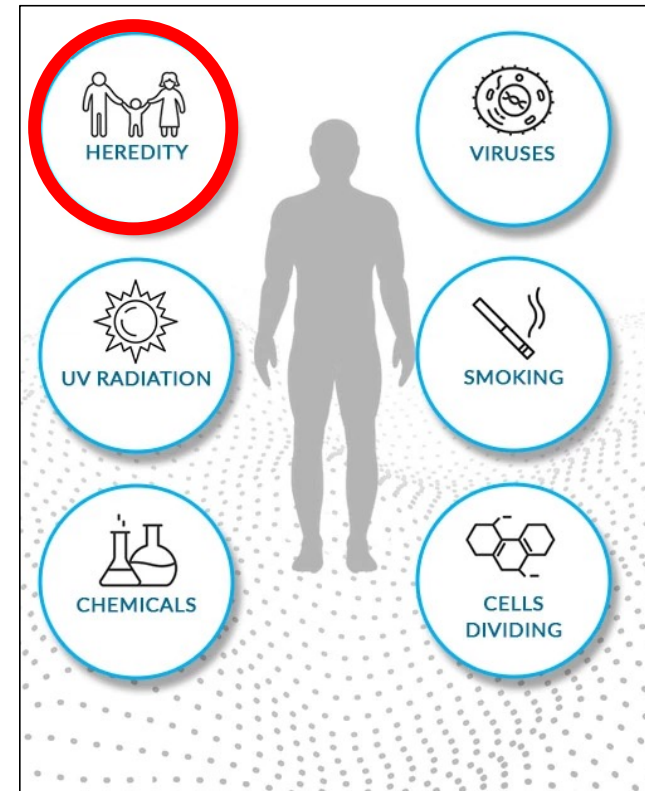
- Large scale (aka, 1000s of individuals) genetic/genomic studies using matched tumor and normal tissues show that Pathogenic Germline Mutations in Highly Penetrant Genes CAUSE Cancer and can do so independent of any other factors (*i.e.*, exogenous exposures to toxicants).
- The initiation and progression of cancers caused by pathogenic germline mutations in these highly penetrant genes are due to **endogenous genetic mechanisms** [*i.e.*, inactivating acquired somatic mutations and/or Loss of Heterozygosity (LOF; *i.e.*, mitotic recombination, gene conversions, and interstitial deletions)], **or due to endogenous metabolic processes and the accumulation of endogenous metabolites** (*i.e.*, formaldehyde, acetaldehyde and methylglyoxal).
- 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 Cause MM in mice and can do so independently of asbestos exposure.
- Highly Penetrant pathogenic null mutations in essential tumor suppressors like *BAP1* and *BRCA1/2* Cause Cancer in Specific Tissues due to Endogenous Genetic and Metabolite specific mechanisms of malignant transformation

# “ALL CANCER IS GENETIC”

Ngeow and Eng, 2016

- All cancers are caused by alterations to the genes in your cells. These are called “gene mutations.”
- When a cell with mutations divides, the mutated genes are passed on to the next generation of cells. Over time, cells can acquire enough mutations to stop working normally. As these cells grow out of control, they can become cancer.
- Because all cancers are caused by gene mutations, all cancers are considered “genetic diseases”.
- Genetic changes that cause cancer **can be inherited or arise from certain environmental exposures**. Genetic changes can also happen because of errors that occur as cells divide.
- For more information: National Cancer Institute (<https://www.cancer.gov/about-cancer/causes-prevention/genetics>)
- Hence, discovering genetic driver mutations using Next Generation Sequencing in individuals with cancer are determinative of treatment in the **Era of Precision Medicine**.

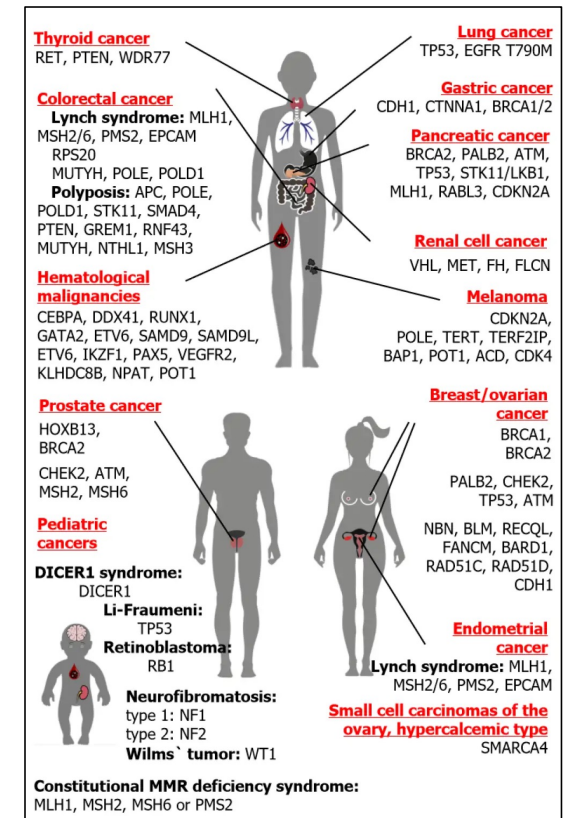
## What Causes Genetic Changes?



Adapted from <https://www.medicover.pl/en/cancers/cancer-genetic-research/>

# 10% of All Cancers are Associated with Hereditary Cancer Predisposition Syndromes (a.k.a. Syndromic Cancers)

- **Cancers caused by an Inherited Mutation are called Hereditary Cancers.** The remaining cancers - those not caused by an inherited mutation - are called sporadic cancers.
- These “Syndromic” Hereditary Cancer Predisposition Syndromes comprise ~10% of ALL malignancies in the general population and are caused by mutations (changes) in certain genes that are passed down from parents to children.
- *“Although the list of these syndromes is growing, other classes of genetic predisposition to cancer are being recognized that do not fit the established criteria. Among these are genetic syndromes presenting cancer as a secondary phenotype, non-syndromic genetic variants influencing tumor development, and polygenic contributions to cancer. This article discusses diverse genomic mechanisms” Aref-Eshghi et al., 2022*



Adapted from Imyanitov et al., 2023



# A New Class of Genes is Emerging that Cause Non-Syndromic Cancers that Do NOT follow Traditional Criteria for Inherited Malignancies

- ***“Evidence is accumulating to support the presence of high penetrant germline mutations in up to 20% of individuals who develop cancer without a syndromic involvement [19,50–53]. Most of these cases do not meet the criteria based on clinical/family history to be eligible for germline genetic testing [50,52].” Aref-Eshghi et al., 2022***
- ***“The growing use of paired tumor-normal paired sequencing is providing an opportunity for the discovery of novel cancer-predisposing genes.” Aref-Eshghi et al., 2022***

Advances in Molecular Pathology 5 (2022) 9–27

## ADVANCES IN MOLECULAR PATHOLOGY

### Hereditary Cancer and Cancer Predisposition Syndromes



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#### KEYWORDS

• Hereditary cancer • Cancer predisposition • Non-syndromic cancer • Polygenic cancer

#### KEY POINTS

- Cancer predisposition syndromes are frequently caused by a germline mutation in a tumor suppressor gene followed by a somatic loss of the functional allele.
- Genetic predisposition can be a feature of many other congenital syndromes with broad etiologies including chromosomal, imprinting, and single-gene defects.
- A new class of genes is emerging that cause non-syndromic cancers that do not always follow the traditional criteria for inherited malignancies.
- The involvement of hereditary factors in most cancers remains to be polygenic or multifactorial.
- The adaptation of genomic and transcriptomic testing in cancer is expected to expand our understanding of hereditary involvement in cancer.

#### INTRODUCTION

Despite being a genetic disease, most incidences of cancer are sporadic with no traceable family history. Occasionally, patients present with signs that raise the suspicion of an underlying hereditary involvement. These include an earlier age of onset, multiple primary tumors, bilateral involvement, rare malignancies, and a constellation of tumors specific to a recognizable syndrome. These so-called cancer predisposition syndromes have been most accountable for inherited malignancies, comprising approximately 10% of the cancer occurrences in the population. Although the list of these syndromes is growing, other classes of genetic predisposition to cancer are being recognized

are genetic syndromes presenting cancer as a secondary phenotype, non-syndromic genetic variants influencing tumor development, and polygenic contributions to cancer. This article discusses diverse genomic mechanisms and diagnostic testing advances in hereditary cancer.

#### CANCER PREDISPOSITION SYNDROMES IN CHILDREN AND ADULTS

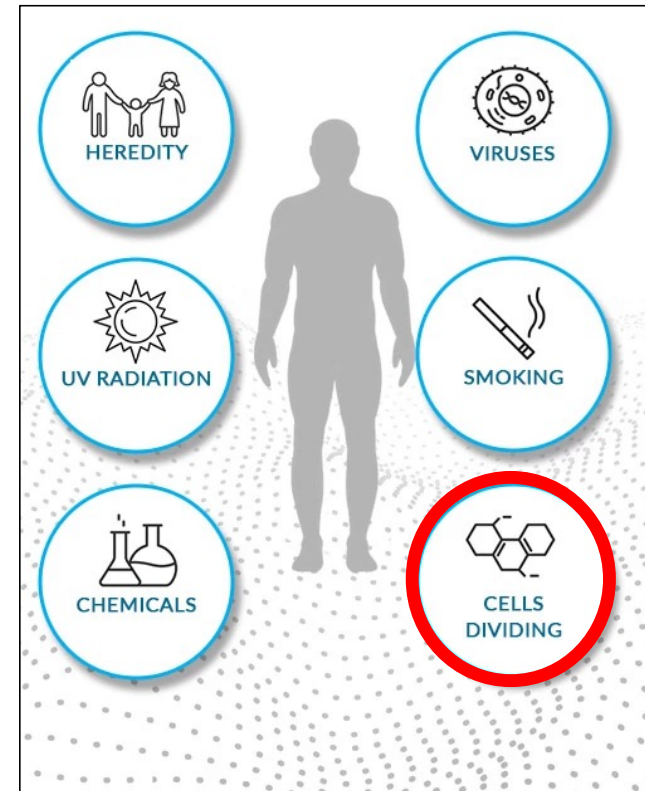
Several hereditary syndromes are characterized by a predisposition to malignancy as the main/only clinical feature. These syndromes are often caused by inactivating mutations in tumor suppressor genes that encode

# “ALL CANCER IS GENETIC”

Ngeow and Eng, 2016

- All cancers are caused by alterations to the genes in your cells. These are called “gene mutations.”
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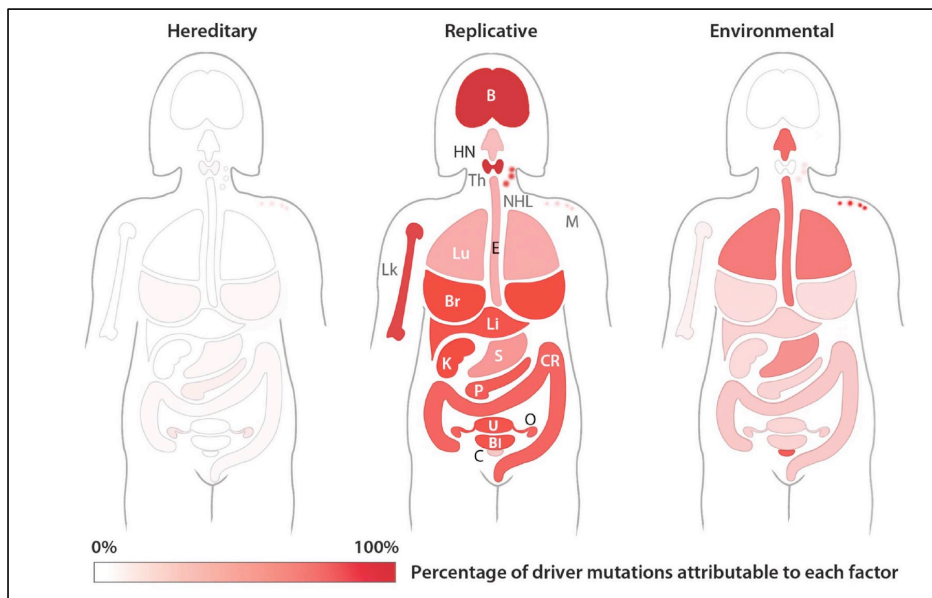
## What Causes Genetic Changes?



Adapted from <https://www.medicover.pl/en/cancers/cancer-genetic-research/>

# Tomasetti and Vogelstein (2015) & Tomasetti *et al.* (2017) DNA Replication Errors in Stem Cells Can Explain the Majority of Cancers

Adapted from Tomasetti *et al.*, 2017

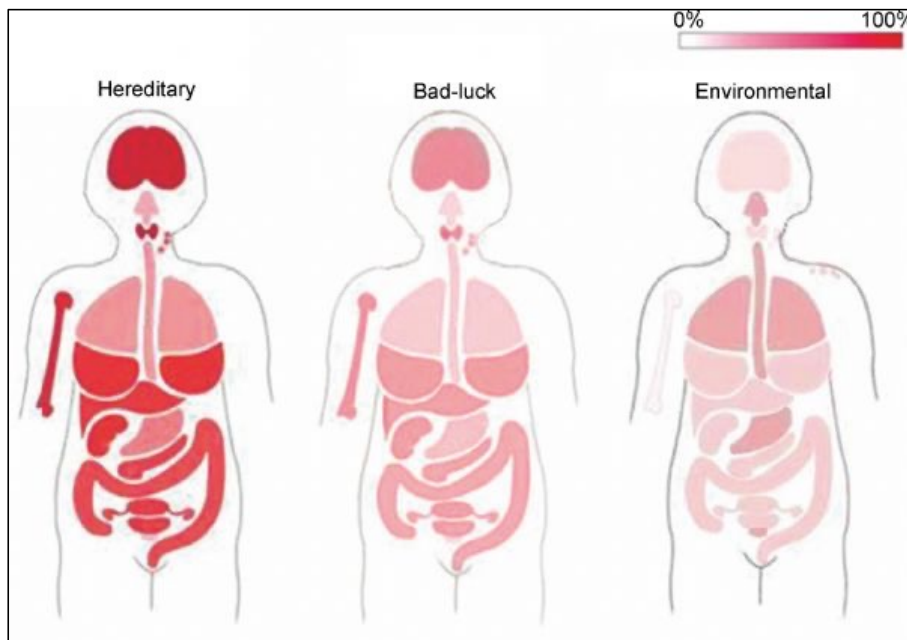


“For each of 18 representative cancer types, the schematic depicts the proportion of mutations that are inherited, due to environmental factors, or due to errors in DNA replication (i.e., not attributable to either heredity or environment). The sum of these three proportions is 100%. The color codes for hereditary, replicative, and environmental factors are identical and span white (0%) to brightest red (100%).” Tomasetti *et al.*, 2017

***“It has been debated for many years whether genetic, extrinsic factors [97] (i.e., exposure to pathogen infection and environmental agents contributes to tumorigenesis) or intrinsic factors [98] (“bad luck hypothesis” — random error mutations during DNA replication in some stem cells could explain the majority of cancer population) contribute to tumorigenesis and metastasis. Although both “bad luck” and “extrinsic factor” hypotheses disagree with each other in terms of intrinsic and extrinsic factors for contributing tumorigenesis, they agree that heredity plays a minimal role in tumorigenesis.” Jiang *et al.*, 2020***

Since 2015 Numerous Large Scale Genomic and Genetic Studies Support Statements made by Jiang *et al.* (2020)

***“...heredity does not play a minimal, but dominant role in tumorigenesis.”***



The degree of contributions for each factor to tumorigenesis and metastasis in each organ (bone, bone marrows, brain, thymus, respiratory tract, lung, stomach, colon, kidney, liver, ovary, and urinary system) is illustrated by the color depth (from light pink to deep red).  
Adapted from Jiang *et al.*, 2020

**“The key limitation of both “bad luck” and “extrinsic factor” hypotheses is that they are cancer cell-centered and ignored the variance of the host immune system. Here we and others have shown that germline pathogenic variants play a significant role in the cancer-immunity system, highlighting that heredity does not play a minimal, but dominant role in tumorigenesis.”** Jiang *et al.*, 2020



# Key Studies Supporting a Dominant Role of Germline Mutations in Tumor Onset and Progression

- **Marty *et al.*, 2017** (this powerful study found that patient specific HLA variants directly determine which oncogenic mutations will pass undetected by the immune system and allow for clonal selection-allow us to predict which HLA genotype will predict which driver mutations are likely to occur in the tumors of specific patients)
- **Waszak *et al.*, 2017 published in Nature, by The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium, 2020** (incredibly diverse and large study that shows that germline variants determine/shape the somatic mutation landscape in cancer). This study systematically evaluated the association of germline variants with somatic mutation signatures in **2642 individuals across 39 cancer types**.
- **Ramroop *et al.*, 2019** (a review that shows germline variation plays an important role in shaping the somatic processes during tumorigenesis)
- **Alexandrov *et al.*, 2020** (repertoire of somatic signatures; more than 50% of all known somatic signatures in cancer are directly determined by germline variants which cause endogenous processes leading to cancer)
- **Demanelis *et al.*, 2020** (telomere length study-ancestry and germline variants plays a major role in determining telomere length; age is the primary factor).
- **Jiang *et al.*, 2020** (germline genomes have a dominant-heritable contribution to cancer immune evasion)
- **Moore *et al.*, 2021** [fascinating study that sequenced 29 different cell types (tissues) within an individual and showed somatic mutational signatures (mostly due to endogenous germline factors);
- **Srinivasan *et al.*, 2021** [large study (**17152 sequenced cancer patients**) shows that highly penetrant pathogenic germline variants cause cancer independent of external factors, and pre-determine mechanisms-specific somatic phenotypes]
- **Kaplanis *et al.*, 2022** [pathogenic germline mutations in DNA-repair genes cause a hypermutation germline phenotype; **massive study having sequenced 21,879 trios (father, mother, child)**]
- **Vali-Pour *et al.*, 2022** [large study (**sequenced 15000 normal tissue and human tumors**) that shows the impact of germline variants on somatic mutation processes]
- **Weinstock *et al.*, 2023** [a massive study (**sequencing whole genomes of 43,693 blood genomes**) that showed that germline variants drive specific somatic mutations signatures in blood cancers]

# Pathogenic Germline Mutations Drive 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).
- **Germline genetic variants play a critical role in shaping the selection of and generation of specific somatic mutations during tumorigenesis** (Ramroop *et al.*, 2019).

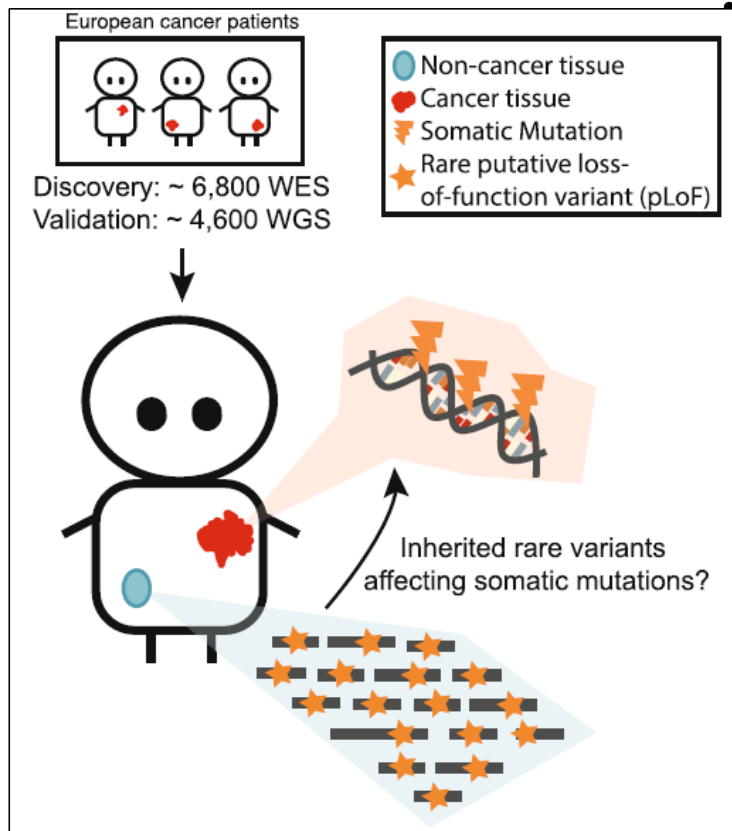
## Pan-Cancer Analysis of Whole Genomes of 2642 Cancer Patients Shows that Rare Germline Variants Determine Somatic Mutation Patterns, Point Mutations, and Structural Variants

- The Waszak *et al.* (2017) was published in Nature by The ICGC/TCGA Pan Cancer Analysis of Whole Genome Consortium in 2020.

- **They concluded:**

**“Common and rare germline variants affect patterns of somatic mutation, including point mutations, structural variants and somatic retro-transposition. A collection of papers from the PCAWG Consortium describes non-coding mutations that drive cancer beyond those in the TERT promoter<sup>4</sup>; identifies new signatures of mutational processes that cause base substitutions, small insertions and deletions and structural variation<sup>5,6</sup>; analyses timings and patterns of tumor evolution<sup>7</sup>; describes the diverse transcriptional consequences of somatic mutation on splicing, expression levels, fusion genes and promoter activity<sup>8,9</sup>; and evaluates a range of more-specialized features of cancer genomes<sup>8,10–18</sup>.”**  
ICGC/TCGA Pan Cancer Analysis of Whole Genome Consortium, 2020

# Rare Germline Variants Shape the Mutation Landscape in Human Somatic Cells



*"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%." Vali-Pour et al., 2022*

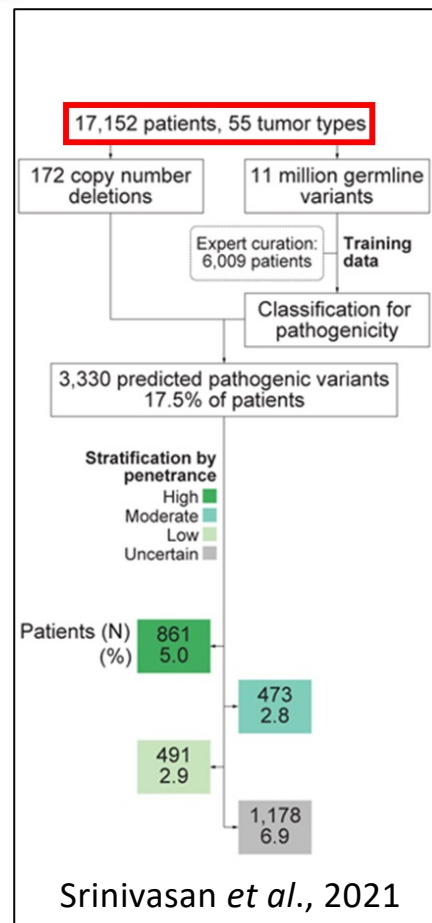
The authors concluded:

***"Rare inherited variants in a diverse set of genes therefore contribute to inter-individual differences in somatic mutation accumulation." Vali-Pour et al., 2022***

***"In conclusion, our findings highlight the role of rare inherited germline variants in shaping the mutation landscape in human somatic cells, leading to variability in somatic mutagenesis between individuals." Vali-Pour et al., 2022***

# Highly Penetrant Pathogenic Germline Mutations Cause Cancer Independent of Other Factors

Large Scale Genetics/Genomics Study by Researchers and Physicians at the Memorial Sloan Kettering Cancer Center



- A study by Srinivasan *et al.* (2021) published in Nature Genetics demonstrated how pathogenic highly penetrant germline mutations cause cancer independent of external factors; and directly cause the loss of the 2<sup>nd</sup> allele 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)
- The authors 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.

## Highly Penetrant Pathogenic Germline Mutations Cause Cancer Independent of Other Factors

*“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

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

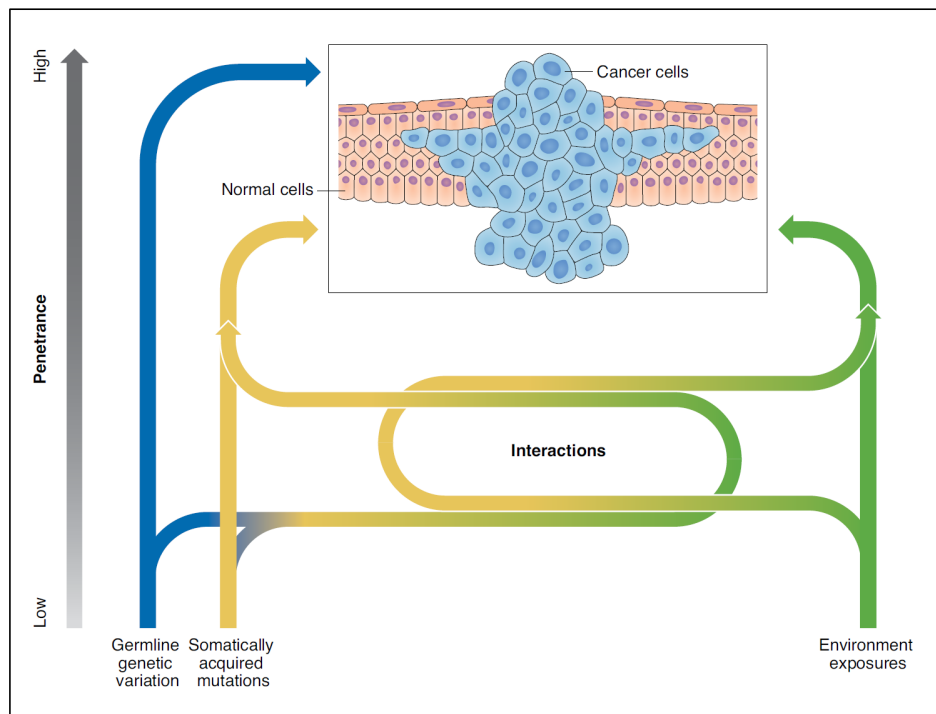
## GERMLINE MUTATIONS

### 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

# 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.”*

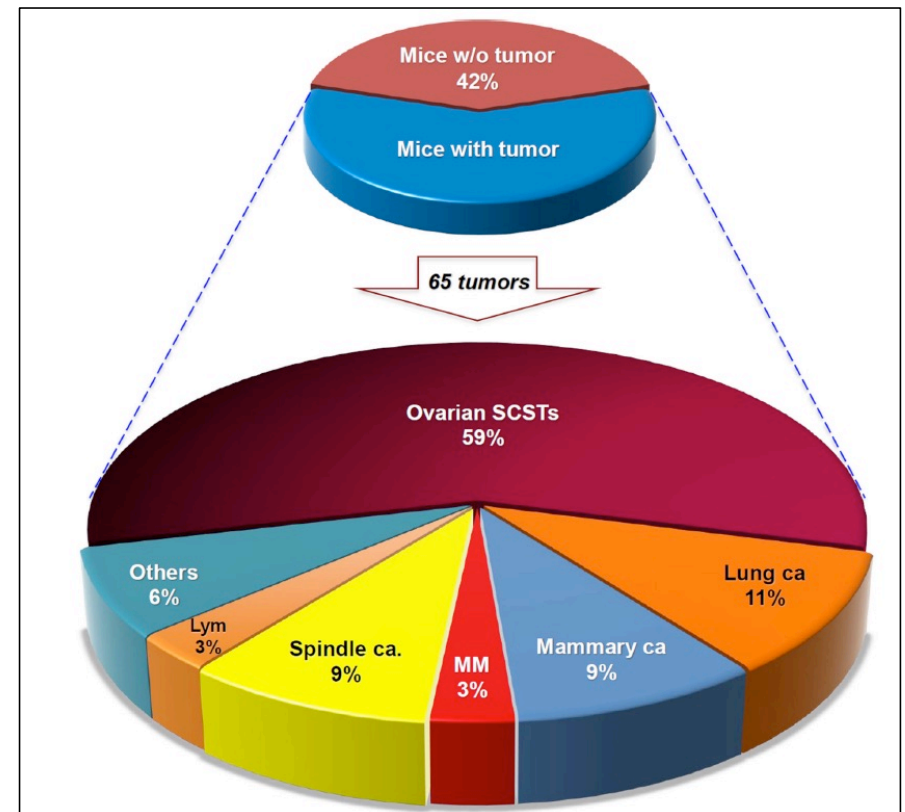
Chanock, 2021; Nature Genetics

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



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

- ***“Collective spectrum of spontaneous tumors observed in all three Bap1-mutant mouse models. Upper pie chart depicts percentages of mice with or without tumors. Lower pie chart indicates percentages of different primary tumors observed. Altogether, neoplastic tumors were identified in 54 of 93 (58%) Bap1-mutant mice. A total of 65 different primary tumors were found in the 54 mice, with 11 mice having two different primary tumors. Mice with bilateral ovarian SCSTs are counted here as one tumor. Percentages of different tumor types refer to proportion of all 65 tumors.”*** Kadariya et al., 2016
- ***“Interestingly, combining the data from all three mouse models (KO, L, and W) revealed that about two-thirds of the Bap1 mutant mice developed spontaneous tumors, with the majority of the tumors comprising ovarian sex cord stromal tumors (SCSTs) (49). Virtually all female mice developed SCST (some bilaterally) within 12–30 months of age.”*** Cheung and Testa, 2017

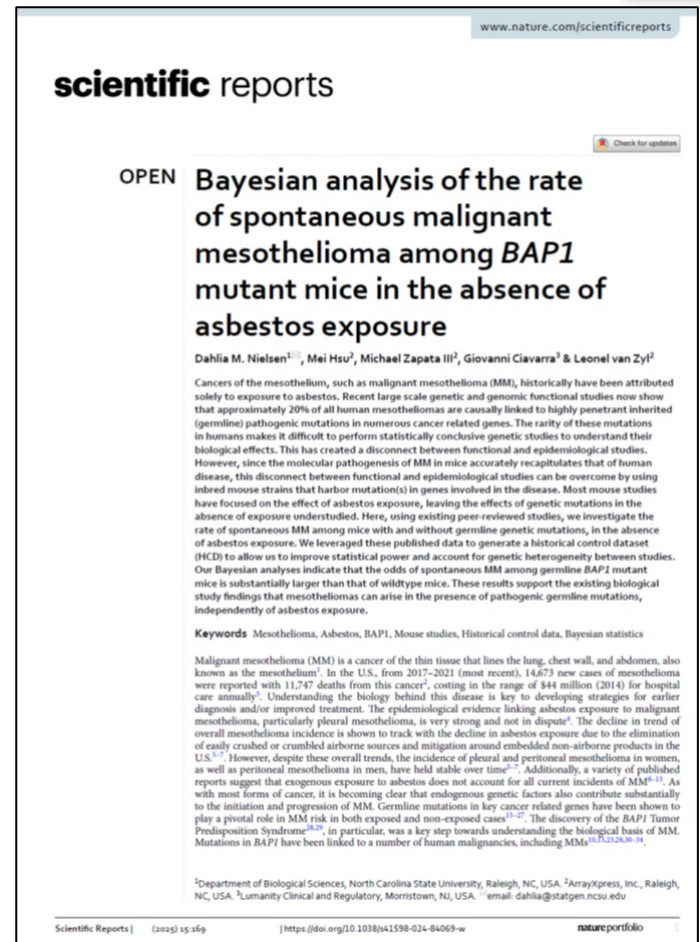


Adapted from Kadariya et al., 2016

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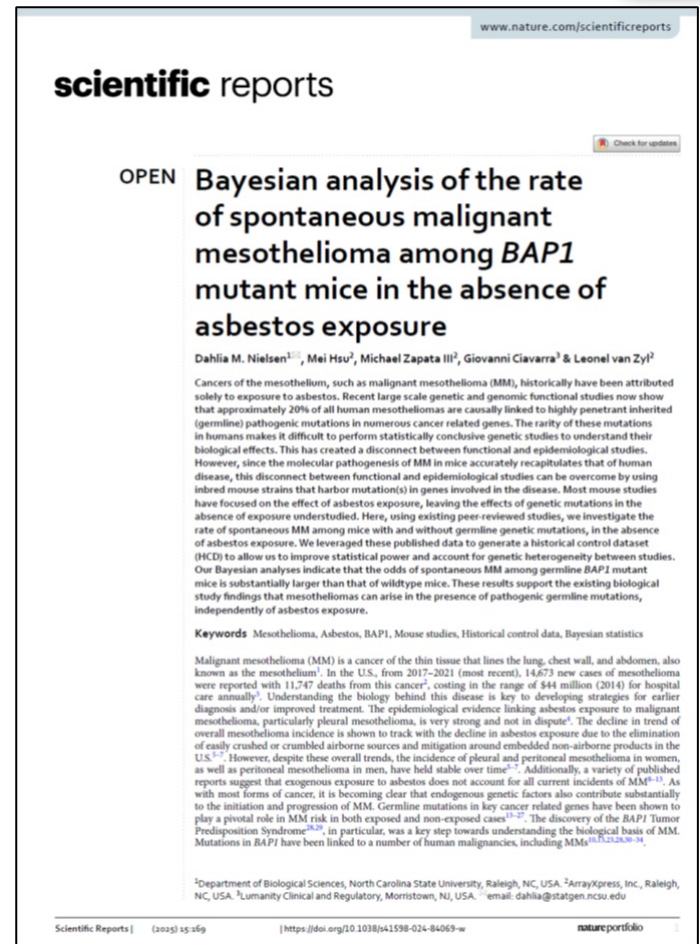
*“In such patients, this germline “driver” was likely the founding event that directly promoted cellular transformation and tumor initiation, ultimately shaping the somatic mutational profile of the resulting tumors, with subsequent somatic driver events arising to accelerate tumor formation, progression,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

- Overall, we 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, we 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***



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

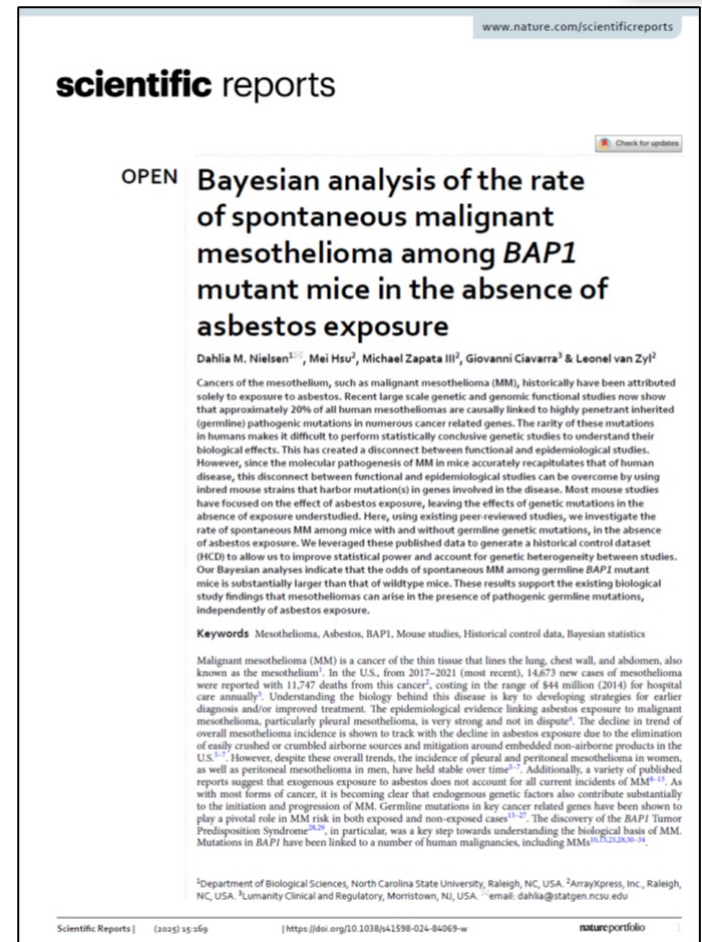
- “Pathogenic mutations in *BAP1* have been shown not only to initiate tumors, but to also drive the subsequent acquisition of endogenous mutations and epigenetic changes<sup>85–90</sup>. Much like *TP53*, studies have demonstrated that *BAP1* functions within the parameters of all three classes of tumor suppressor genes: as gatekeeper (directly regulating cell cycle control and cell proliferation); as caretaker (regulating genomic stability); and as landscaper (regulating chromatin modification)<sup>30,32,91,92</sup>. 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<sup>28,31,32,93–96</sup>. Nielsen *et al.*, 2025





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

- “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.” Nielsen *et al.*, 2025
- “Numerous published scientific and review studies have demonstrated that up to 20% of MMs are caused by inherited pathogenic mutations in highly penetrant cancer-related genes<sup>16,22,26,31,59</sup>. Some studies estimate MM rates even as high as 36%<sup>27</sup>.” Nielsen *et al.*, 2025
- “Germline null mutations in several highly penetrant tumor suppressor genes, such as *BAP1* and *TP53*, demonstrate ~100% cancer penetrance<sup>30–32,80</sup>, underscoring that some highly penetrant mutations are themselves sufficient to cause cancer<sup>81</sup>. Pathogenic germline null mutations in the *BAP1* gene have been empirically shown to cause MM and other cancers without exogenous exposures<sup>5–8,23,24,26,31,32,36,37,80,82,83</sup>.” Nielsen *et al.*, 2025



# Genetic Mechanisms for Tissue Specificity in BAP1 Cancer Syndrome

**“Malignancies arising from mutation of tumor suppressors have unexplained tissue proclivity. For example, BAP1 encodes a widely expressed deubiquitinase for histone H2A, but germline mutations are predominantly associated with uveal melanomas and mesotheliomas. We show that BAP1 inactivation causes apoptosis in mouse embryonic stem cells, fibroblasts, liver, and pancreatic tissue but not in melanocytes and mesothelial cells. Ubiquitin ligase RNF2, which silences genes by monoubiquitinating H2A, promoted apoptosis in BAP1-deficient cells by suppressing expression of the pro-survival genes Bcl2 and Mcl1. In contrast, BAP1 loss in melanocytes had little impact on expression of pro-survival genes, instead inducing Mitf. Thus, BAP1 appears to modulate gene expression by countering H2A ubiquitination, but its loss only promotes tumorigenesis in cells that do not engage an RNF2-dependent apoptotic program.”** He et al., 2019

## RESEARCH

### CANCER

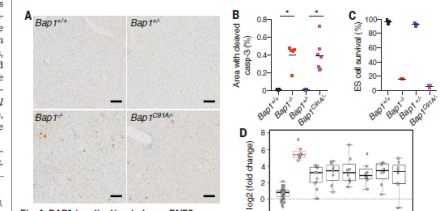
#### Intrinsic apoptosis shapes the tumor spectrum linked to inactivation of the deubiquitinase BAP1

Meng He<sup>1</sup>, Mira S. Chaudhry<sup>1,2</sup>, Joshua D. Webster<sup>2</sup>, Sarah Kummerfeld<sup>1,2</sup>, Rohit Raja<sup>1</sup>, Subira Chandhury<sup>1</sup>, Ying-Jin Chen<sup>3</sup>, Zora Modrusan<sup>4</sup>, Benjamin Haley<sup>4</sup>, Debra L. Dugger<sup>1</sup>, Jeffrey Eastham-Anderson<sup>2</sup>, Shari Lau<sup>1</sup>, Anwesha Dey<sup>5</sup>, Roger Caithien<sup>6</sup>, Merone Roese-Girma<sup>7</sup>, Kim Newton<sup>1,2</sup>, Vishva M. Dixit<sup>1,2</sup>

Malignancies arising from mutation of tumor suppressors have unexplained tissue proclivity. For example, BAP1 encodes a widely expressed deubiquitinase for histone H2A, but germline mutations are predominantly associated with uveal melanomas and mesotheliomas. We show that BAP1 inactivation causes apoptosis in mouse embryonic stem cells, fibroblasts, liver, and pancreatic tissue but not in melanocytes and mesothelial cells. Ubiquitin ligase RNF2, which silences genes by monoubiquitinating H2A, promoted apoptosis in BAP1-deficient cells by suppressing expression of the pro-survival genes Bcl2 and Mcl1. In contrast, BAP1 loss in melanocytes had little impact on expression of pro-survival genes, instead inducing Mitf. Thus, BAP1 appears to modulate gene expression by countering H2A ubiquitination, but its loss only promotes tumorigenesis in cells that do not engage an RNF2-dependent apoptotic program.

Heterozygous germline mutations in the deubiquitinase BAP1 cause a human cancer predisposition syndrome with a high incidence of uveal melanoma, mesothelioma, and renal cell carcinoma (1–5). Consistent with BAP1 being a classic two-hit tumor suppressor gene, patient tumors often exhibit inactivating somatic mutation of the remaining functional BAP1 allele (1–5). BAP1 deficiency may combine with other oncogenic mutations to promote tumorigenesis because BAP1 is often mutated with BRCA1 in melanoma (6, GNAQ) or RNF2 in uveal melanoma (7) and CDKN2A or NF1 in mesothelioma (8). Mice with heterozygous Bap1 mutations also have an increased tumor incidence, including spontaneous and asbestos-induced mesotheliomas (9–11). Combined deletion of Bap1, Nf2, and Cdkn2a in the mouse thymic cavity produces mesothelioma with features of the human disease (12). In other studies, Bap1 deletion from mouse melanocytes synergized with mutant BRAF and ultraviolet light exposure to produce melanoma (13), and Bap1 heterozygosity in nephron progenitor cells combined with VHL loss to cause renal cell carcinoma (14). Therefore, BAP1 mediates tumor suppression in both mice and humans.

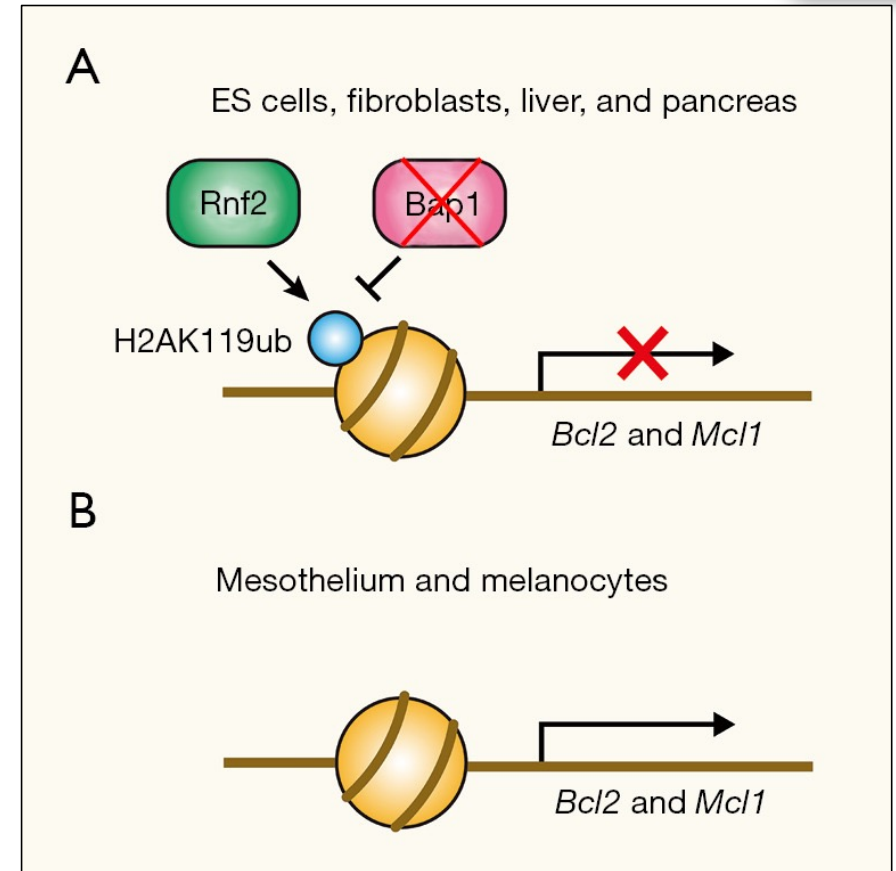
To gain insights into how BAP1 suppresses tumorigenesis, we generated knock-in mice (Rosa26-CreER<sup>2</sup> Bap1<sup>fl/fl</sup>) that express catalytically inactive BAP1 Cys<sup>28</sup>→Ala (C9A) instead of wild-type BAP1 after tamoxifen treatment (fig. S1A). Bap1<sup>fl/fl</sup> embryonic stem (ES) cells derived from these mice expressed amounts of BAP1 C9A comparable to those of wild-type BAP1 in Bap1<sup>+/+</sup> control ES cells (fig. S1B). Tamoxifen-induced expression of BAP1 C9A in adult mice caused splenomegaly, leukocytosis, neutropenia, anemia, thrombocytopenia, liver damage, and atrophy of the pancreas within 4 weeks (fig. S1, C to H), as does tamoxifen-induced BAP1 deficiency (15, 16). Therefore, BAP1 enzymatic activity is critical for normal hematopoiesis and normal functioning of the liver and pancreas.



**Fig. 1. BAP1 inactivation induces RNF2-dependent apoptosis.** (A) Rosa26-CreER<sup>2</sup> ES cells immunolabeled for cleaved caspase-3 (green) at 4 weeks after tamoxifen treatment. Scale bars, 100  $\mu$ m. (B) Quantification of the immunolabeling in (A). Each symbol represents a different mouse. Lines indicate the mean.  $^{**}P < 0.01$  by Student's *t* test ( $n = 5$  to 7 mice per genotype). (C) Graph indicates the percentage of viable Rosa26-CreER<sup>2</sup> ES cells at 6 days after beginning 4-OHT treatment. Each symbol represents a different mouse. Lines indicate the mean.  $^{**}P < 0.01$  by Student's *t* test ( $n = 5$  to 7 mice per genotype). (D) Graph indicates gRNA (gRNA) enrichment in ES cells surviving Bap1 deletion. Each dot represents a distinct gRNA. Ntc, nontargeting control.

# Genetic Mechanisms for Tissue Specificity in *BAP1* Cancer Syndrome

***“The authors propose that this difference in the engagement of Rnf2 and Bap1 in the regulation of the pro-survival gene expression determines the cellular fate after Bap1 inactivation. When a cell spontaneously loses functional BAP1 through LOH in BAP1 mutation carriers, that precancerous cell vanishes due to apoptotic cell death in the majority of tissues. In mesothelial and melanocytic tissues, however, such a precancerous cell with inactive BAP1 would persist because apoptosis is not triggered in those cell types. Having survived BAP1 loss, those precancerous cells in melanocytic tissue and mesothelium are poised to respond to additional tumor-promoting effects of BAP1 inactivation.”*** Machida, 2019



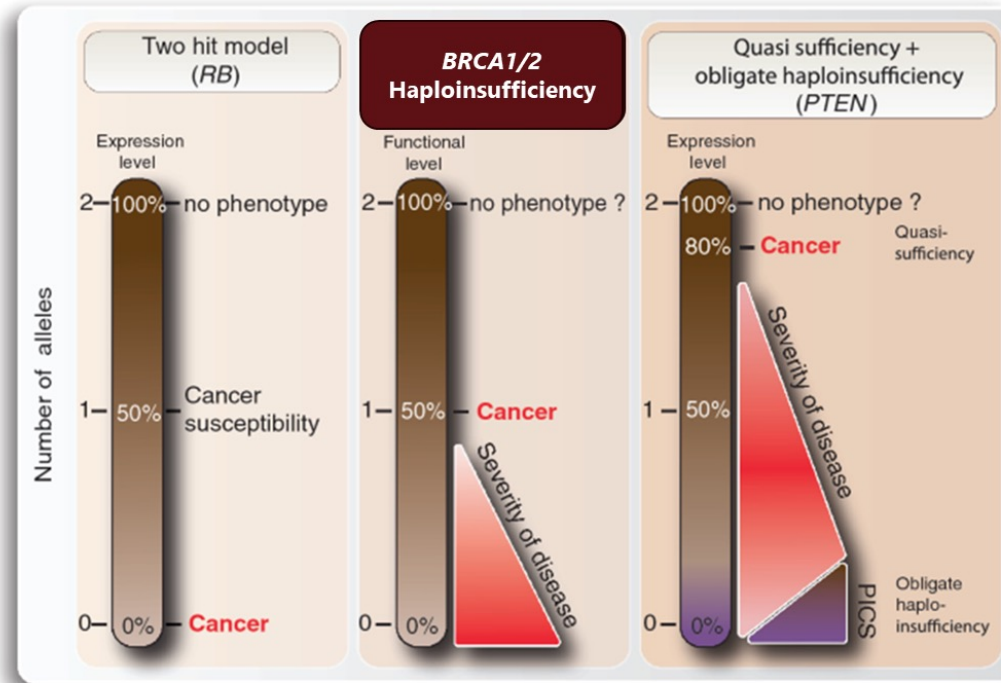
## Mono-Allelic (Heterozygous) *BRCA1/2* Inactivation Suffices for Carcinogenesis

*“Recent results from genomic studies on large collections of cancers arising in human *BRCA1* and *BRCA2* mutation carriers have provided supporting evidence that a significant fraction of cancers arising in mutation carriers do not undergo LOH, and therefore retain a functional, expressed wild-type copy.”* Venkitaraman, 2019

*“In cancers from different tissues that bear germline *BRCA* gene mutations, there was no evidence for LOH (either through genetic alterations or at the level of RNA expression) in ~37% of *BRCA1*-mutant tumors, and in ~53% of *BRCA2*-mutant tumors, suggesting that a functional copy of the wild-type allele has been retained in a significant fraction.”* Venkitaraman, 2019

*“Collectively therefore, these studies provide several lines of clinical genetic evidence that the *BRCA* genes need not always conform to the Knudson “two-hit” paradigm, in that mono-allelic heterozygous mutations affecting *BRCA1* or *BRCA2* suffice for tumour formation in varying contexts.”* Venkitaraman, 2019

# BRCA1/2 Haploinsufficiency



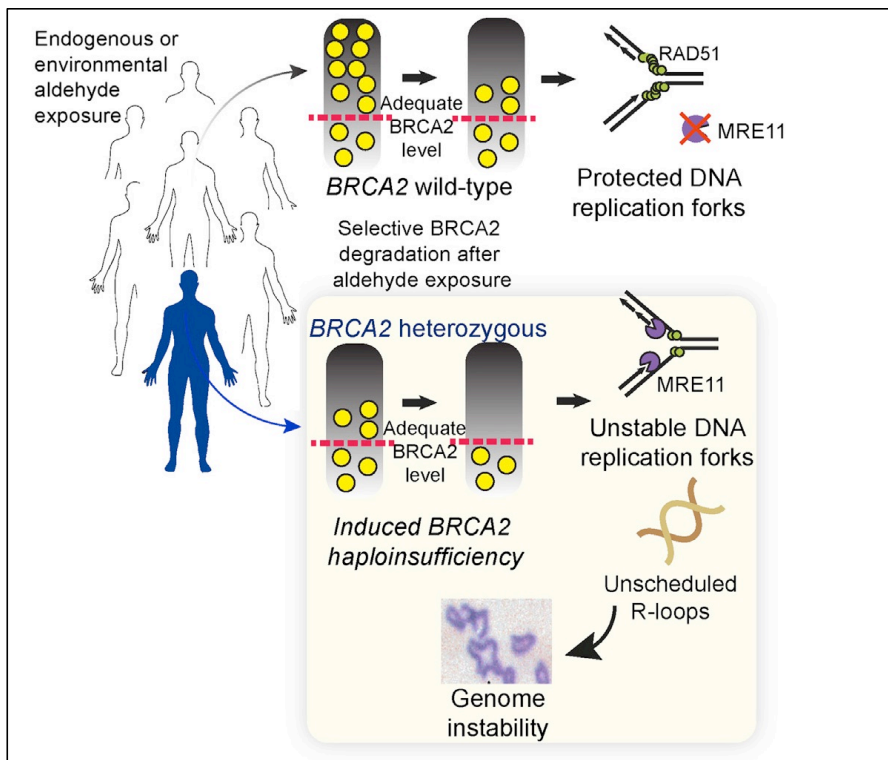
A brown gradient represents a continuum of expression related to the number of alleles present (black numbering). **Left, the two-hit paradigm as exemplified by the tumor suppressor, RB. Loss of one allele induces cancer susceptibility; loss of two alleles induces cancer. Middle, classical haploinsufficiency in BRCA1/2. Loss of one allele is sufficient for induction of cancer.** Right, quasi-sufficiency and obligate haploinsufficiency. Quasi-sufficiency refers to the phenomenon whereby tumor suppression is impaired after subtle expression downregulation without loss of even one allele. Obligate haploinsufficiency occurs when TSG haploinsufficiency is more tumorigenic than complete loss of the TSG, usually due to the activation of fail-safe mechanisms following complete loss of TSG expression. Adapted from Carlberg, C., Velleuer, E. (2021). Tumor Suppressor Genes and Cell Fate Control. In: Cancer Biology: How Science Works. Springer, Cham. [https://doi.org/10.1007/978-3-030-75699-4\\_3](https://doi.org/10.1007/978-3-030-75699-4_3)



# Tissue-Specific Carcinogenesis in *BRCA1/2* Mutation Carriers

*“Why BRCA gene mutations should predispose to cancers in specific tissues, when their physiological functions relevant to tumor suppression seem ubiquitous, remains a major unresolved question. Several recent lines of evidence touched upon in the foregoing open new lines of investigation that may help to address this issue.”* Venkitaraman, 2019

# Aldehyde-Induced *BRCA1/2* Haploinsufficiency Drives Genomic Instability and Malignant Transformation



Adapted from Tan *et al.*, 2017

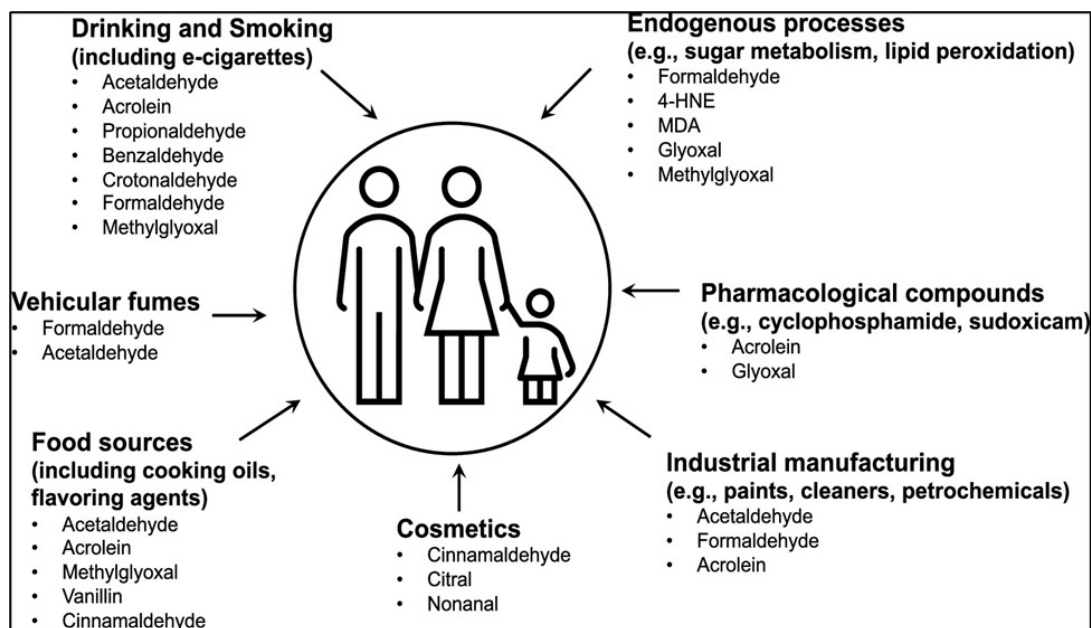
*“...endogenous metabolites like aldehydes... through cellular metabolism in different tissues, are able to trigger DNA damage, induce *BRCA2* haploinsufficiency accompanied by the outgrowth of genomically unstable progeny (Tan et al., 2017), or even cell death (Tacconi et al., 2017) in *BRCA*-mutant cells. Aldehyde accumulation may in this way modify tissue-specific cancer progression in *BRCA* mutation carriers.” Venkitaraman, 2019*

## Exposure to Formaldehyde and Acetaldehyde Potentiate Spontaneous Mutagenesis in Pathogenic BRCA1/2 Germline Carriers

*“Our findings suggest a new model for carcinogenesis in individuals who carry germline mutations truncating a single copy of BRCA2. Exposure to aldehydes like formaldehyde or acetaldehyde, which are both widespread in our environment and also accumulate endogenously in certain tissues, could potentiate spontaneous mutagenesis in the cells of mutation carriers, predisposing to cancer”*

Tan *et al.*, 2017

# Common Environmental and Endogenous sources of Toxic Aldehydes



Adapted from Vijayraghavan and Saini, 2023

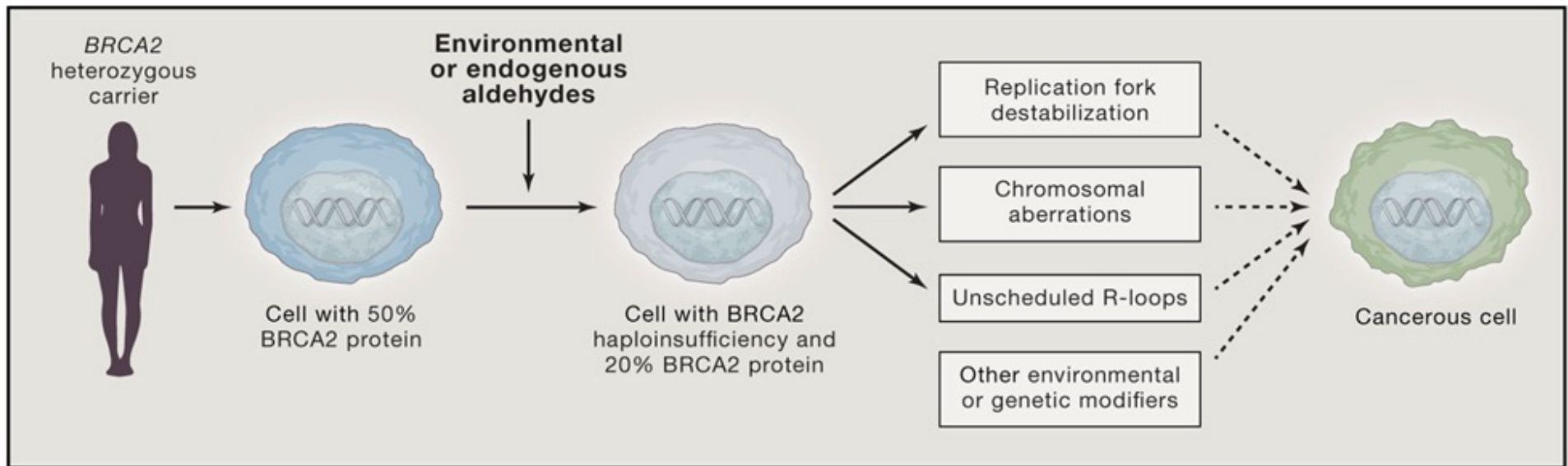
Aldehydes are widespread in the environment, with multiple environmental (external) sources such as cigarette smoke, alcohol consumption, food and beverages, industrial effluents, and additives.

Aldehydes are also endogenously synthesized as normal byproducts of daily cellular metabolism in mammals. The toxic effects of exposure to aldehydes have been observed in numerous studies (Reingruber and Pontel, 2018).

At the molecular level, aldehydes damage DNA by forming DNA Interstrand-Crosslinks (ICLs), DNA and protein adducts, lead to lipid peroxidation, and are associated with significant increased risk of developing cancer (Friedenson, 2011; Parmar and D'Andrea, 2017; Vijayraghavan and Saini, 2023).

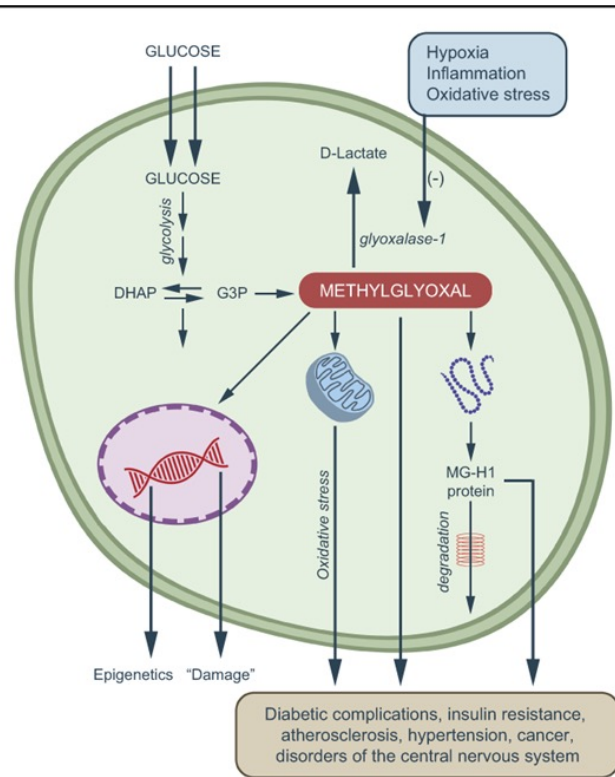
As a result, aldehyde exposure, especially from environmental (exogenous) and endogenous (byproduct of metabolism) sources, poses a high risk to human health (Seitz and Stickel, 2010; Friedenson, 2011; Inci *et al.*, 2013; Parmar and D'Andrea, 2017; Reingruber and Pontel, 2018; Garaycochea *et al.*, 2018; Vijayraghavan and Saini, 2023)

## Endogenous Aldehydes Deplete *BRCA1/2* Protein, Disable its Function, and Induce Genomic Instability & Tumorigenesis in *BRCA1/2* Heterozygous (Monoallelic) Cells



***"BRCA2 protein is susceptible to aldehyde-mediated degradation. In cells from normal individuals with wild-type BRCA2, the reduction in BRCA2 protein levels after aldehyde exposure do not fall below the threshold of adequacy. In BRCA2 heterozygous carriers [like Mr. DuBois], however, BRCA2 protein levels are already reduced by 50%, and aldehyde exposure causes further reduction (to 20% of wild-type levels), resulting in "induced haploinsufficiency" for BRCA2. Aldehyde-induced BRCA2 haploinsufficiency provokes replication fork instability, formation of RNA-DNA hybrids (R-loops), and spontaneous chromosomal aberrations. These aldehyde-induced anomalies then trigger carcinogenesis in BRCA2 heterozygous carriers."*** Parmar and D'Andrea, 2017 (emphasis added)

# Accumulation of Methylglyoxal (MGO) in Type 2 Diabetes Patients Drives the Pathogenesis of Cancer and Other Diabetic Complications

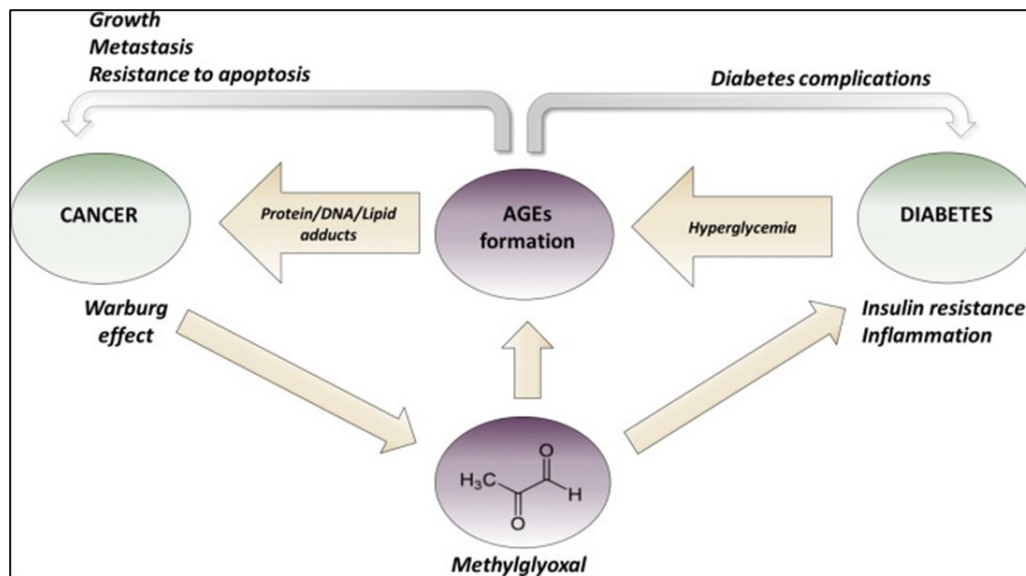


Adapted from Schalkwijk and Stehouwer, 2020

***“The formation and accumulation of methylglyoxal (MGO), a highly reactive endogenous aldehyde, has been shown to drive the pathogenesis of type 2 diabetes, vascular complications of diabetes, and several other age-related chronic inflammatory diseases such as cardiovascular disease, cancer, and disorders of the central nervous system” (Schalkwijk and Stehouwer, 2020).***

***“MGO is mainly formed as a byproduct of glycolysis and, under physiological circumstances, detoxified by the glyoxalase system. MGO is the major precursor of nonenzymatic glycation of proteins and DNA, subsequently leading to the formation of advanced glycation end products (AGEs). MGO and MGO-derived AGEs can impact on organs and tissues affecting their functions and structure” (Schalkwijk and Stehouwer, 2020).***

# Increased Formation and Accumulation of MGO in Type 2 Diabetes Patients Drives the Pathogenesis of Cancer and Other Diabetic Complications



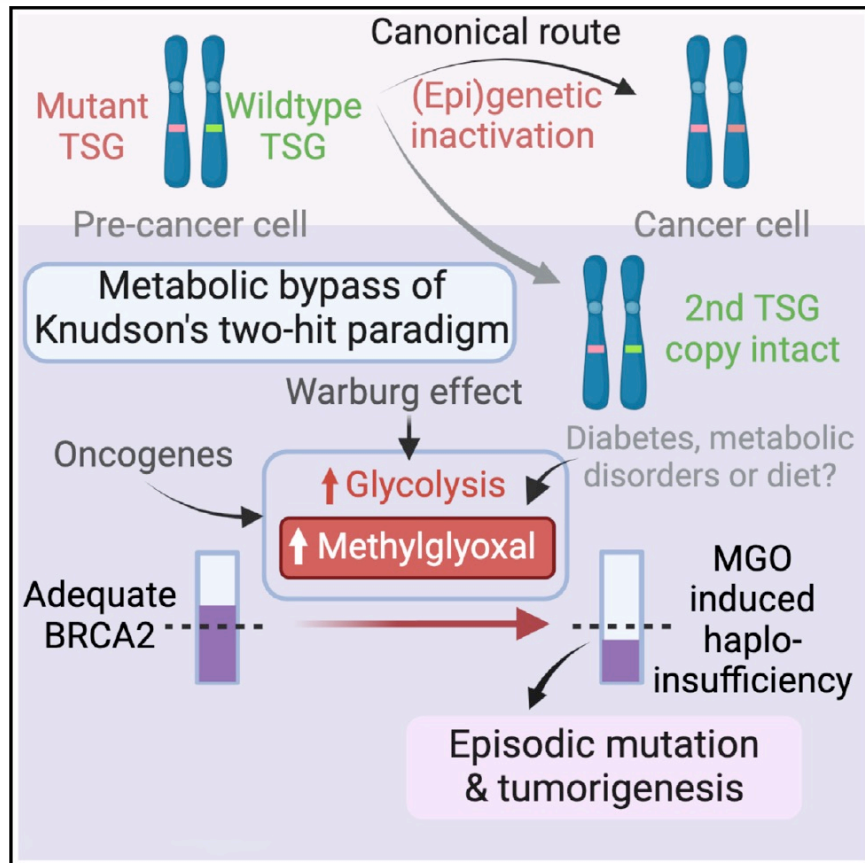
Adapted from Bellier et al., 2019

*"Advanced Glycation Endproducts (AGEs) formation as the link between diabetes and cancer where methylglyoxal (MGO), a potent precursor of AGEs, sustains a vicious cycle. Diabetic hyperglycemia favors the formation of MGO and AGEs that will create a favorable environment for growth, metastasis, progression and therapy resistance on the one hand, and to diabetes chronic complications on the other hand."* Bellier et al., 2019

*"Hyperglycemia primarily supports cancer cells' increased energetic and biosynthetic needs. High glucose circulating levels also trigger direct and indirect mechanisms notably leading to the induction of insulin, growth factors and inflammatory cytokines secretion all of which favor cancer cell proliferation, migration and resistance to apoptosis."* Bellier et al., 2019



# Aldehyde Induced (MGO) Transiently Inactivates *BRCA2* Function, Bypassing Knudson's "Two-hit" Requirement



Methylglyoxal

***"MGO transiently inactivates *BRCA2* function, bypassing Knudson's "two-hit" requirement"***

***"Oncogenic or metabolic changes that increase MGO via glycolysis provoke similar events"***

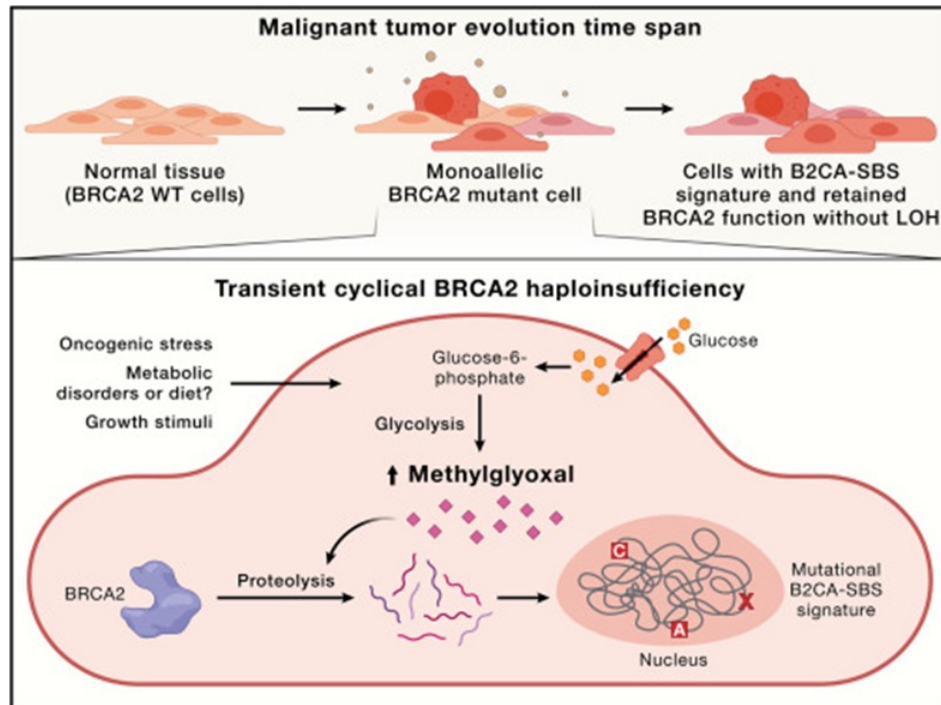
***"This mechanism links metabolic reprogramming, metabolic disorders, or diet to cancer initiation"***

Kong *et al.*, 2024

Adapted from Kong *et al.*, 2024



# High Chronic Intercellular MGO Bypasses Need for Knudson's Two-Hit to Initiate Oncogenesis in BRCA2 Null Germline Mutation Carriers

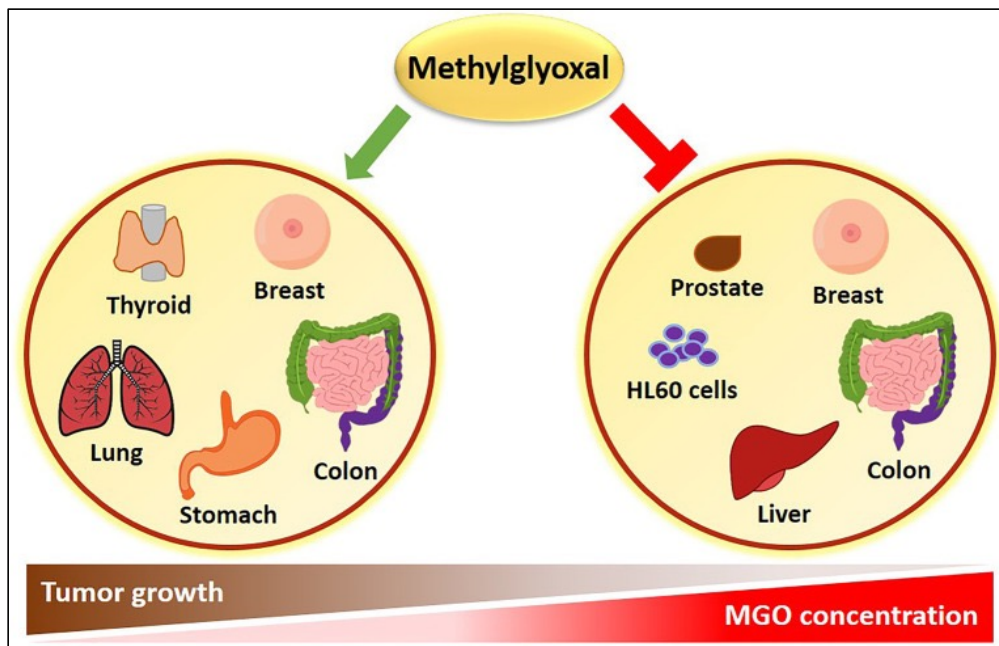


Adapted from Jiang, 2024

*"In cells harboring monoallelic BRCA2 truncations, methylglyoxal (MGO) accumulates when glycolysis is activated in response to oncogenic stress or certain metabolic disorders [Type 2 Diabetes Mellitus; T2DM]. Prolonged exposure to MGO can trigger BRCA2 proteolysis, temporarily disabling BRCA2's tumor suppressor functions and inducing episodic SBS mutations without permanent BRCA2 inactivation, leading to functional haploinsufficiency and promoting cancer genome evolution."* Jiang, 2024 (emphasis added)

*"Altogether, Kong et al. reveal that BRCA2 degradation induced by a glycolytic metabolite [MGO] connects aerobic glycolysis to the episodes of cancer genome evolution that initiate or maintain tumorigenesis, which may provide insights for future studies on metabolic alterations and tumor evolution."* Jiang, 2024 (emphasis added)

# Tumors Characteristically Associated with Change in Cellular and Intracellular MGO Accumulation



Adapted from Leone *et al.*, 2021

*"Cancer is a disease characterized by uncontrolled growth and proliferation of abnormal cells. Due to lack of oxygen, tumors primarily rely on the anaerobic metabolism of glucose, which has been called the 'Warburg effect.' To compensate for this inefficient energy supply, tumors show a higher rate of glucose uptake and glycolysis. An important consequence is intracellular formation of MGO (56). To survive, cancer cells with such a high glycolytic rate require a high rate of detoxification of MGO, and high expression of Glo1 is a likely mechanism for survival and growth of tumors with high glycolytic rates and related high fluxes of formation of MGO."* Schalkwijk and Stehouwer, 2020

*"Thus increased expression of Glo1 plays a crucial role in the survival and proliferation of tumor cells by lowering MGO concentrations. In addition, Glo1 can directly activate NF- $\kappa$ B and activator protein-1 (AP-1), which results in activation of the PI3K/Akt pathway, promoting cell survival and proliferation (29, 238)." Schalkwijk and Stehouwer, 2020*

## Implications for *BRCA1/2* Carcinogenesis and Public Health

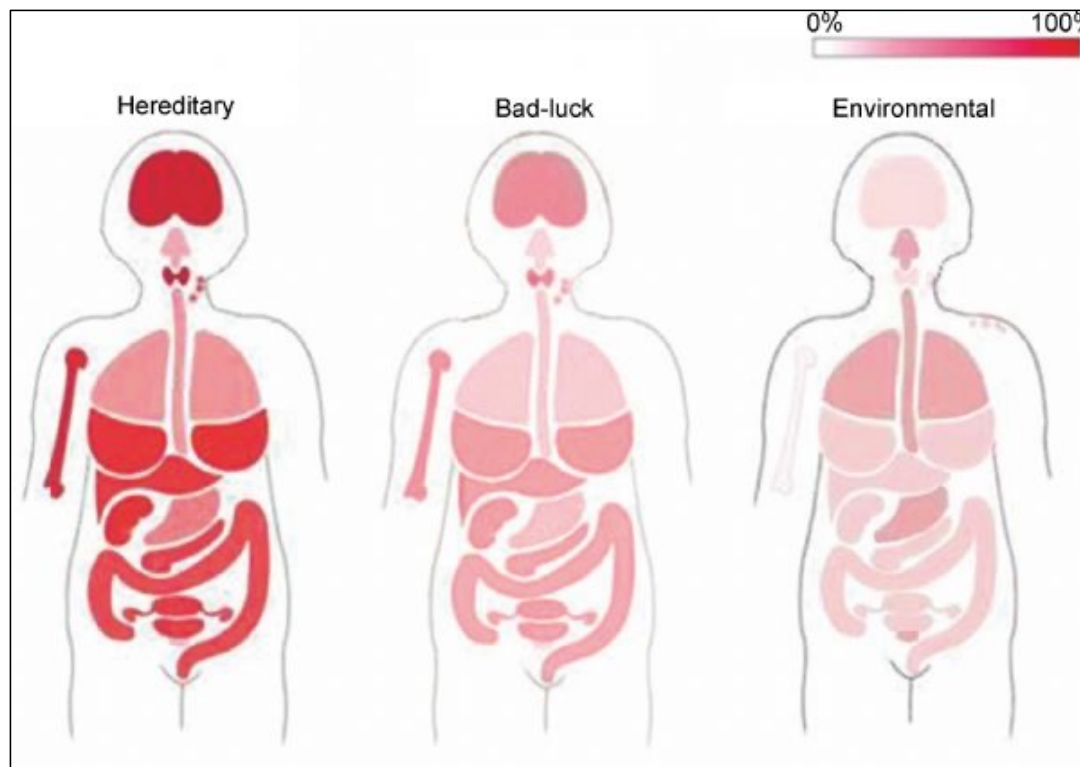
*“In this model, the risk of carcinogenesis among mutation carriers may depend not only on the nature of their germline *BRCA2* mutation and its susceptibility to aldehyde-induced haploinsufficiency but also upon other genetic and environmental factors that determine exposure to aldehydes. Because *BRCA2* mutation carriers typically develop cancers in certain epithelial tissues including the breast, ovary, pancreas, or prostate, we speculate that organ-specific differences in endogenous aldehyde accumulation or extrinsic exposure may account in part for the observed tissue selectivity”* Tan et al., 2017

## Researchers and Physicians at MSK Propose a Fundamental Shift in Current Approaches Related to Clinical Cancer Assessment

- *“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., 2021

# Heredity Plays A Dominant Role in Carcinogenesis

*“...heredity does not play a minimal, but dominant role in tumorigenesis.”*



The degree of contributions for each factor to tumorigenesis and metastasis in each organ (bone, bone marrows, brain, thymus, respiratory tract, lung, stomach, colon, kidney, liver, ovary, and urinary system) is illustrated by the color depth (from light pink to deep red). Adapted from Jiang *et al.*, 2020

# Overall Conclusions

- Large scale (aka, 1000s of individuals) genetic/genomic studies using matched tumor and normal tissues show that Pathogenic Germline Mutations in Highly Penetrant Genes CAUSE Cancer and can do so independent of any other factors (*i.e.*, exogenous exposures to toxicants).
- The initiation and progression of cancers caused by pathogenic germline mutations in these highly penetrant genes are due to **endogenous genetic mechanisms** [*i.e.*, inactivating acquired somatic mutations and/or Loss of Heterozygosity (LOF; *i.e.*, mitotic recombination, gene conversions, and interstitial deletions)], **or due to endogenous metabolic processes and the accumulation of endogenous metabolites** (*i.e.*, formaldehyde, acetaldehyde and methylglyoxal).
- 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 Cause MM in mice and can do so independently of asbestos exposure.
- **Highly Penetrant pathogenic null mutations in essential tumor suppressors like BAP1 and BRCA1/2 Cause Cancer in Specific Tissues due to Endogenous Genetic and Metabolite specific mechanisms of malignant transformation**





# Current Status of Acquiring and Utilizing Genomic Data



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# Current Status of Acquiring and Utilizing Genomic Data

- Refresher on Types of Genetic Data
- Genetic Data in the Medical Records and Other Sources
- Sources of the Genetic Results
- Types of Testing and Sequencing Performed by the Common Clinical Providers
- Getting the Underlying Data
- Annotation of the Data
- New Sequencing
- Disease Specific Panel Development
- The Process of Determining Which Path to Take



# Refresh on Types of Genetic Data

- Germline vs. Somatic
- Exome vs. Whole Genome
- Report vs. Data
- Varying File formats

# 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
  - Due to legacy issues much of the genetic testing we see has been on exome
  - That testing also is often on Somatic (disease) tissue instead of Germline (inherited) tissue
- Often the reports in the medical records only have a limited amount of data either due to outdated protocols or limited information provided to the treating physicians (they dumb it down)
  - Limited coverage by exome sequencing instead of genome sequencing
  - A limited set of genes that were part of an outdated “panel”
  - A subtraction methodology that is residual from an outdated practice of only focusing on pure somatic gene variants
- **There almost always is much more existing data that can be obtained from the commercial sequencing business and already exists with no new sample collection required**
- This is not data that document collection services understand, know what to look for, or can handle

# Sources of the Genetic Results

- First, you have to know it is there
  - Medical Records
  - Depositions
  - Genealogy
- Reports may readily exist within the discovery information
- Clinical providers (Invitae, Tempus, etc)
- Hospital systems more frequently have facilities
- Core labs at university research hospitals
- Research studies often have “off book” testing

# Types of Testing and Sequencing

The content of the data can be a wealth of information, but it varies based on methodology

- Whole Genome Sequencing (Panels)
  - If the underlying technology is WGS, there is a lot of information unreported
  - If it is whole genome sequencing and it includes germline (inherited) tissue, you have significant information for a detailed and QUANTITATIVE scientific analysis
  - If it is whole genome and only contains somatic (involved) tissue, there are still many things that can be estimated or interpreted to produce a working scientific hypothesis
- Exome Sequencing (Panels)
  - If the underlying technology is exome, there still could be highly informative data from both germline and somatic tissue
  - The limitation will be that it is only on coding regions and may miss the majority of variants
- PCR or Assay Based Panels
  - Extremely limited and unlikely to yield information

# Getting the Underlying Data

## 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, then 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
- Subpoenas and **working with the vendors**
- **Focus on the data**, not the provider

# Making Value From the Data

- Annotation of the Data
- Data That Can Be Derived
  - VAF is an example of the information that is usually found but not always reported
  - Specific locations on the variants for databases and predictive scoring
  - CADD scores
  - Many times, VUSs are not reported but are in the data and can work in combinations
  - Penetrance, microsatellite instability, tumor burden, etc. is often discoverable

# Case Examples

- The limitation of Exome Sequencing

- An example of the shortcomings of exome sequencing arose in the Roberto Sotomayor case in Los Angeles
- There, Judge Seigle granted a defense motion to compel exome sequencing of the BAP1 gene
- The lab (Fulgent) ran the sequencing but did not find a mutation in the exome portion of the gene
- As shown in the screenshot below, Fulgent noted that its results did NOT rule out a mutation in the remainder of the gene that was not sequenced

- The benefits of mining the unseen data behind the report

- In this case, the medical records included a standard clinical report that was non informative
- A subpoena to a commercial sequencing service produced a complete digital file of all data generated from sequencing of hundreds of genes
- Review of the data revealed the presence of a highly pathogenic combination of mutations not noted in the "paper" report of the sequencing results

Final Report

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TEST PERFORMED

**Single Gene - Gene**

(1 Gene Panel: BAP1; gene sequencing with deletion and duplication analysis)

---

RESULTS:

No clinically significant sequence or copy-number variants were identified in the submitted specimen.

A negative result does not rule out the possibility of a genetic predisposition nor does it rule out any pathogenic mutations of the sort not queried by this test or in areas not reliably assessed by this test.

---

INTERPRETATION:

Notes and Recommendations:

- Gene specific notes and limitations may be present. See below.
- These results should be interpreted in the context of this individual's clinical findings, biochemical profile, and family history.

# New Sequencing

- **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, saliva 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



# Disease Specific Panel Development

- As a scientific team we want to know all the facts
- We understand the legal environment and it is not our place to make the decisions, just advise/recommend
- You cannot predict which specific genes, but if needed, you can narrow to a “panel” based on literature
- Privacy can be managed by limiting the scope of the data reported to the expert(s)
- Expert reports can and are limited - even when working with WGS, our case reports focus only on disease related genes
  - To genes, proteins, chromosomal translocations, or other genomic variables that experts in genomics and predictive software recognize as certainly, likely, or possibly involved in carcinogenesis and progressions of cancers and can also focus only on pathogenic/likely pathogenic factors in genes with small insertions, small deletions, single nucleotide substitutions, large gene and chromosomal deletions, and/or chromosomal translocations
  - Such restrictions enable maximum scientific certainty concurrently with protecting privacy.
- The process of building a disease specific panel from the literature

# The Decision Process

- First, SCREENING, no matter which side of the courtroom you are on
  - A minority of cases are supported in the literature to be purely genetically driven
  - Conduct a detailed medical records and family history review – a lifelong look, not just since diagnosis
  - Not all cases will support current genetic selection criteria or have support in the literature
- Do the Research
  - Deep dive on records and genealogy to build the timeline and pedigree (exceptions to the rule)
  - Evaluate the literature
- Determine the Path and Type of Reporting
  - Moves from consulting to expert witnessing
  - Varies by jurisdiction and available time

# 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), 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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