ToxicoGenomica

Highly Penetrant Pathogenic Germline Mutations Drive Malignant Transformation Independent of External Factors in a Tissue Specific Manner

Len van Zyl, Ph.D.

10 April 2025

ARRAYXPRESS
Your Contract Research Partner for Next Generation Genomics

Overall Conclusions

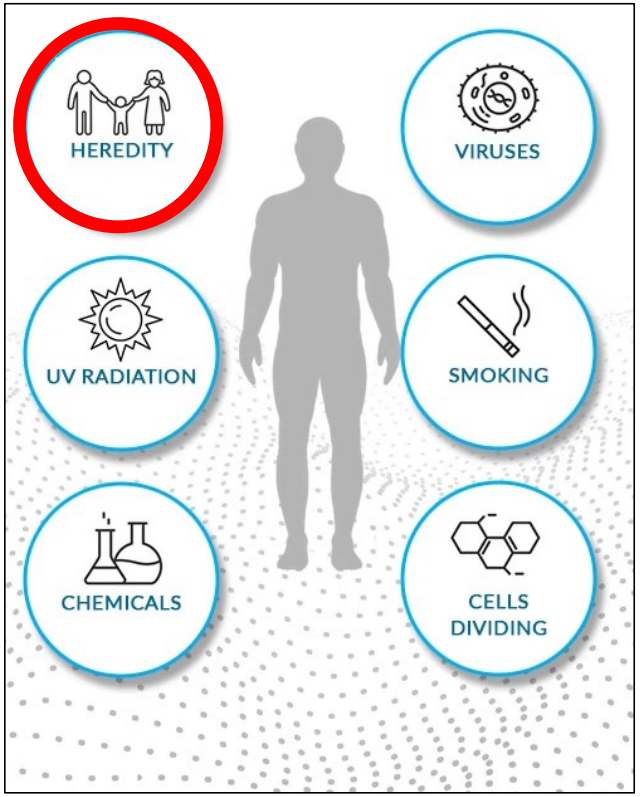
- **Large scale (aka, 1000s of individuals) genetic/genomic studies using matched tumor and normal tissues show that Pathogenic Germline Mutations in Highly Penetrant Genes CAUSE Cancer and can do so independent of any other factors (*i.e.*, exogenous exposures to toxicants).**
- **The initiation and 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.
- Fore 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**.

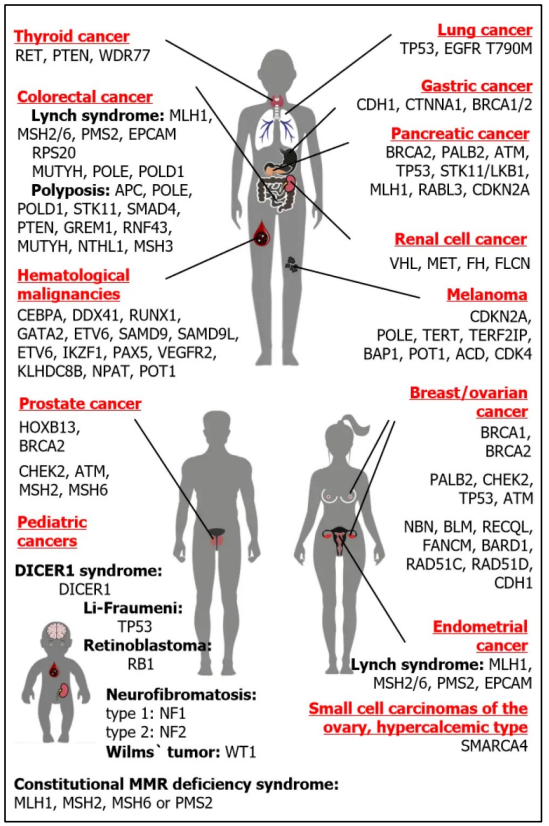
What Causes Genetic Changes?



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)

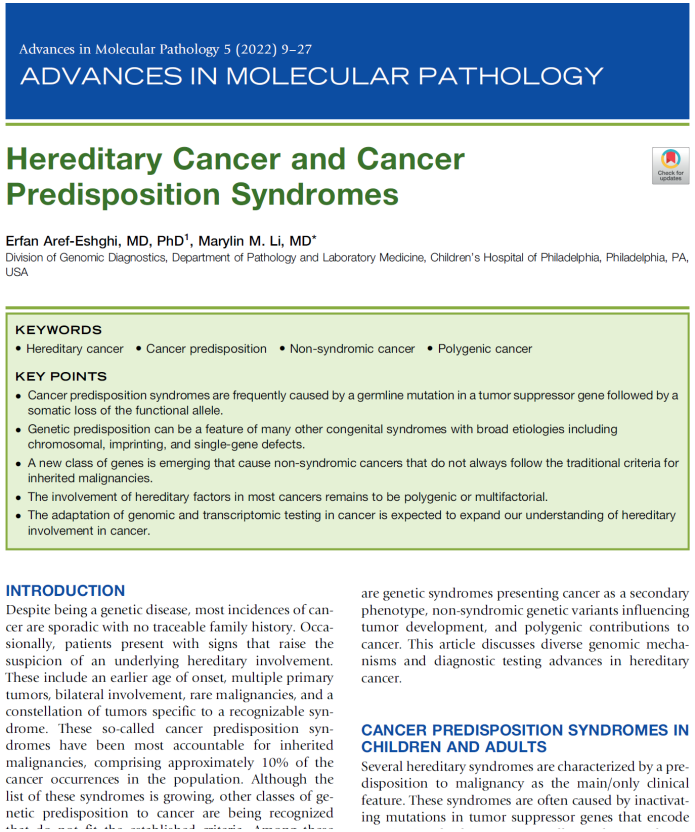
- **Cancers caused by an Inherited Mutation are called <u>Hereditary Cancers</u>.** The remaining cancers - those not caused by an inherited mutation - are called sporadic cancers.
- **These "Syndromic" Hereditary Cancer Predisposition Syndromes comprise ~10% of ALL malignancies in the general population 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

Erfan Aref-Eshghi, MD, PhD[1], Marylin M. Li, MD*
Division of Genomic Diagnostics, Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia, PA, USA

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

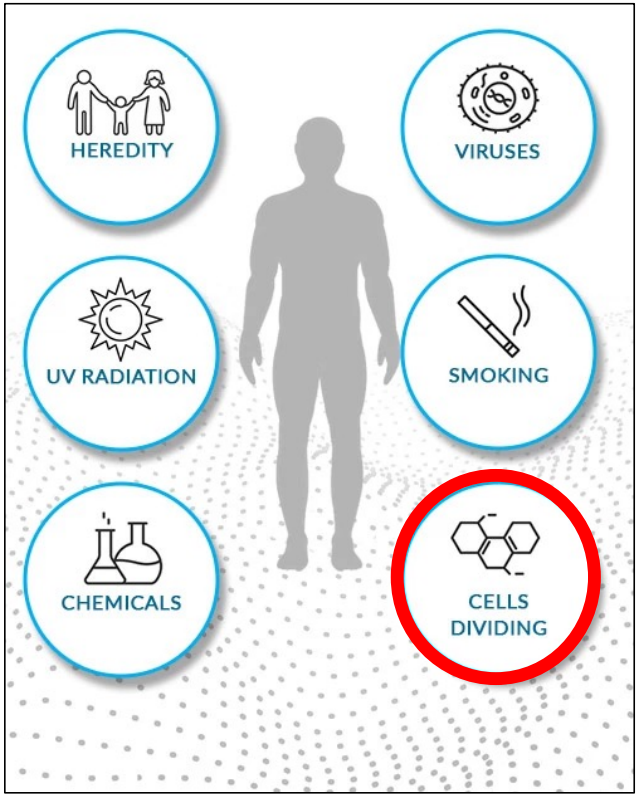
Several hereditary syndromes are characterized by a predisposition to malignancy as the main/only clinical feature. These syndromes are often caused by inactivating mutations in tumor suppressor genes that encode

"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."
- 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.
- Fore 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**.

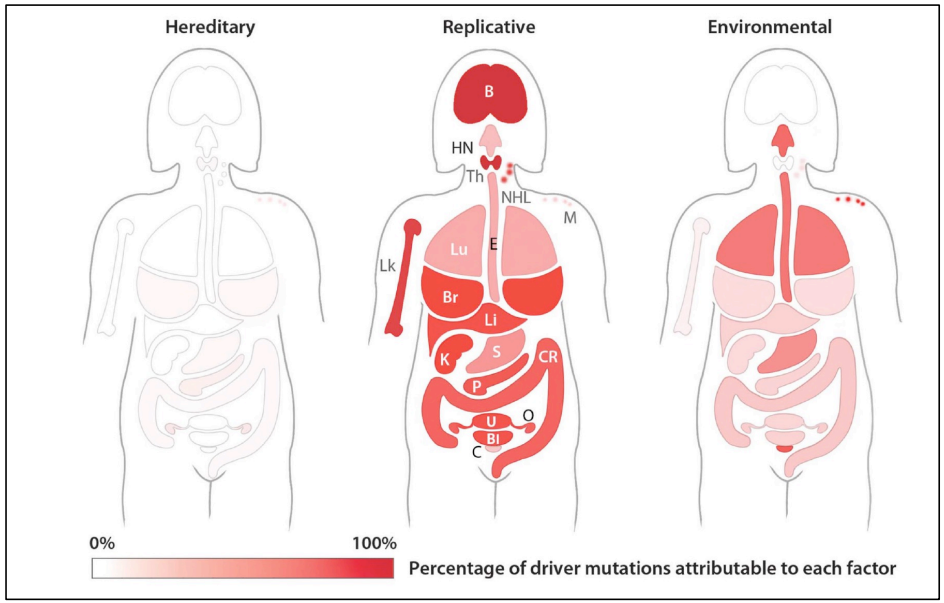
What Causes Genetic Changes?



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

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

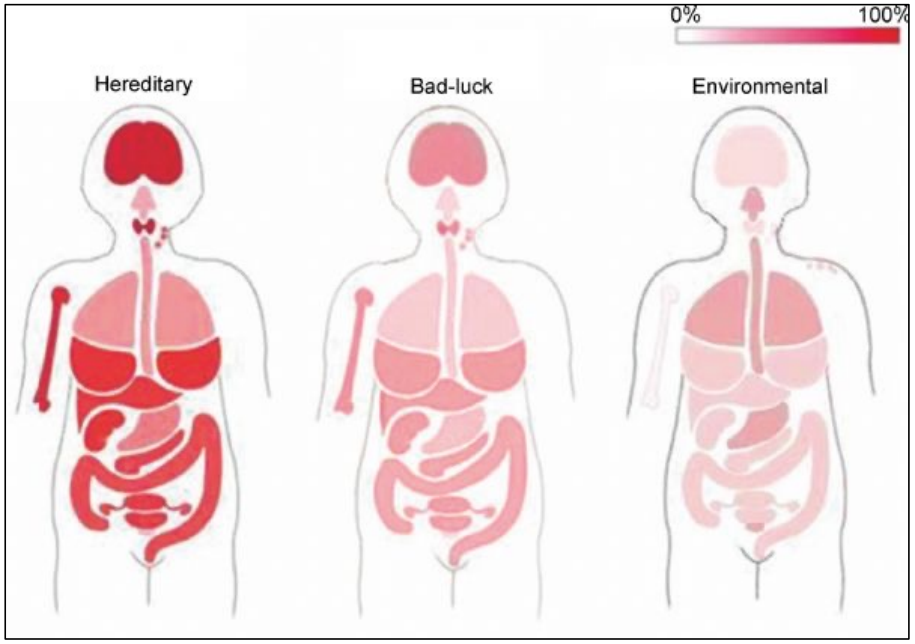


"For each of 18 representative cancer types, the schematic depicts the proportion of mutations that are inherited, due to environmental factors, or due to errors in DNA replication (i.e., not attributable to either heredity or environment). The sum of these three proportions is 100%. The color codes 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).

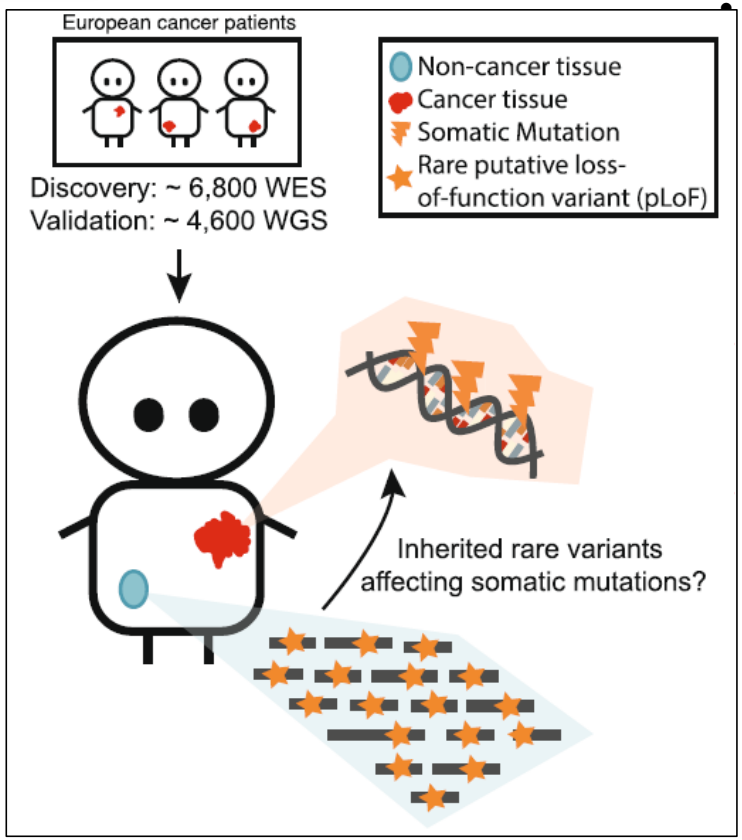
Ramroop *et al*., 2019

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[4]; identifies new signatures of mutational processes that cause base substitutions, small insertions and deletions and structural variation[5,6]; analyses timings and patterns of tumor evolution[7]; describes the diverse transcriptional consequences of somatic mutation on splicing, expression levels, fusion genes and promoter activity[8,9]; and evaluates a range of more-specialized features of cancer genomes[8,10–18]."
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

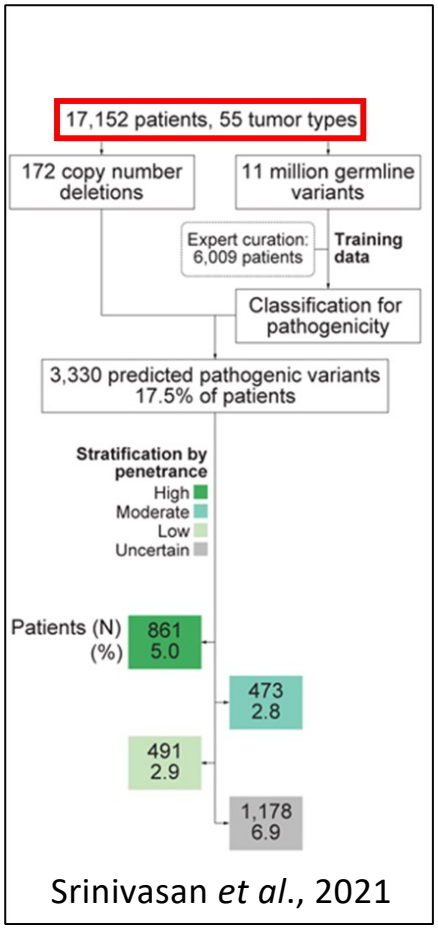
The authors concluded:

"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

Vali-Pour *et al.*, 2022

Highly Penetrant Pathogenic Germline Mutations Cause Cancer Independent of Other Factors



Srinivasan *et al.*, 2021

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 2nd 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**

Srinivasan *et al*., 2022

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

GERMLINE MUTATIONS

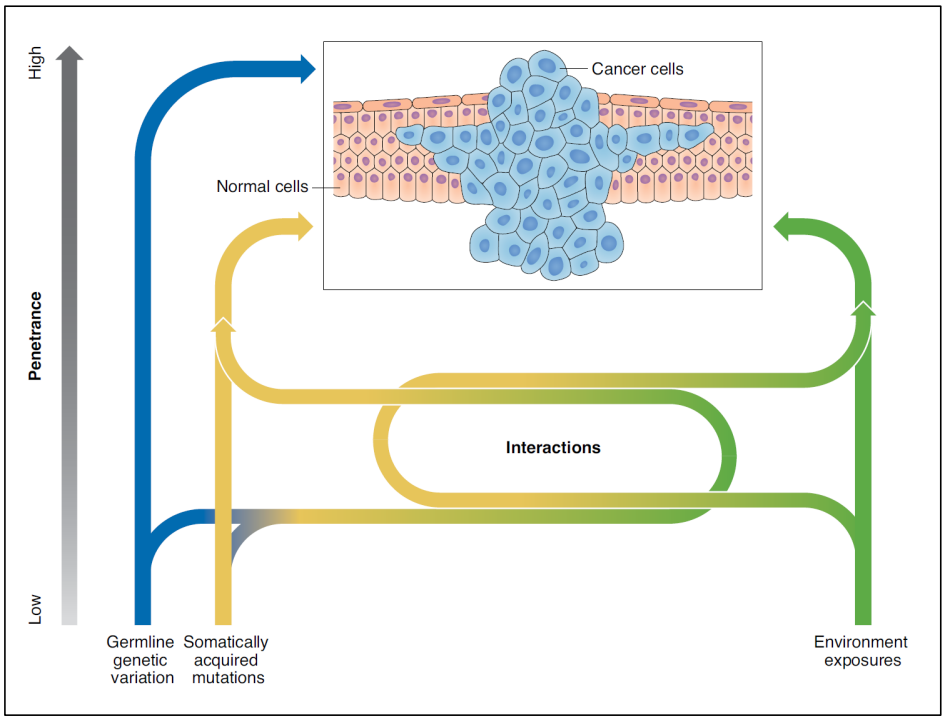
How the germline informs the somatic landscape

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:

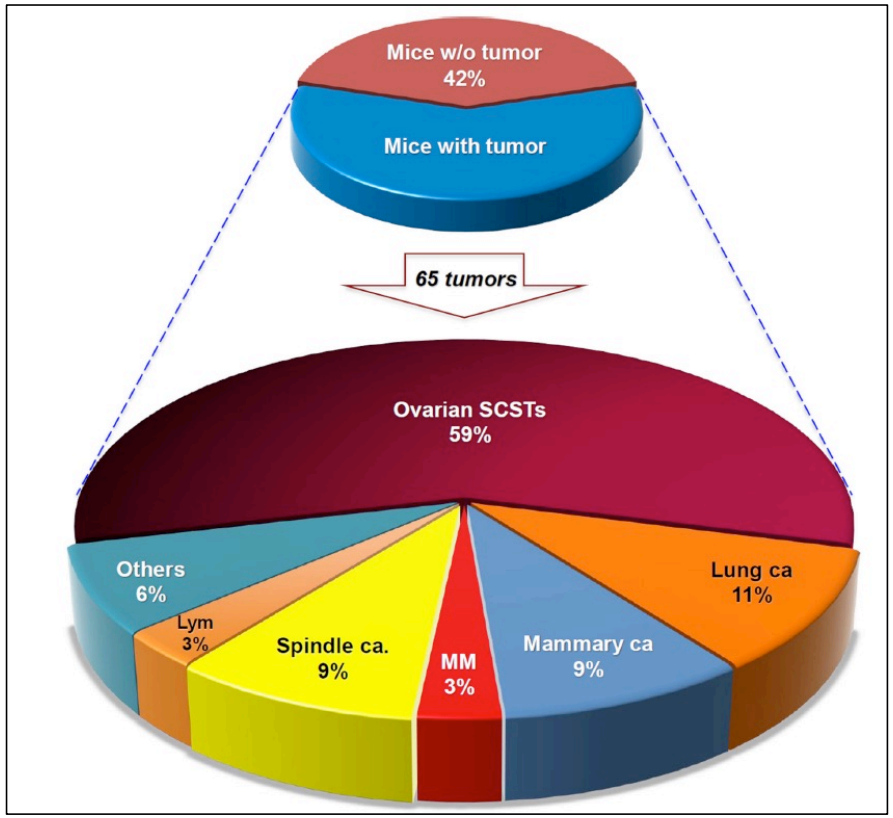
"Tumor development depends on complex interactions between germline variants (dark blue), somatically acquired variants (yellow) and environmental exposures (green). Penetrance also has a modifying role in which highly penetrant (germline) variants are more likely to drive tumorigenesis without the influence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

Chanock, 2021; Nature Genetics

Adapted from Chanock, 2021; based on data generated from a massive genomics study 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

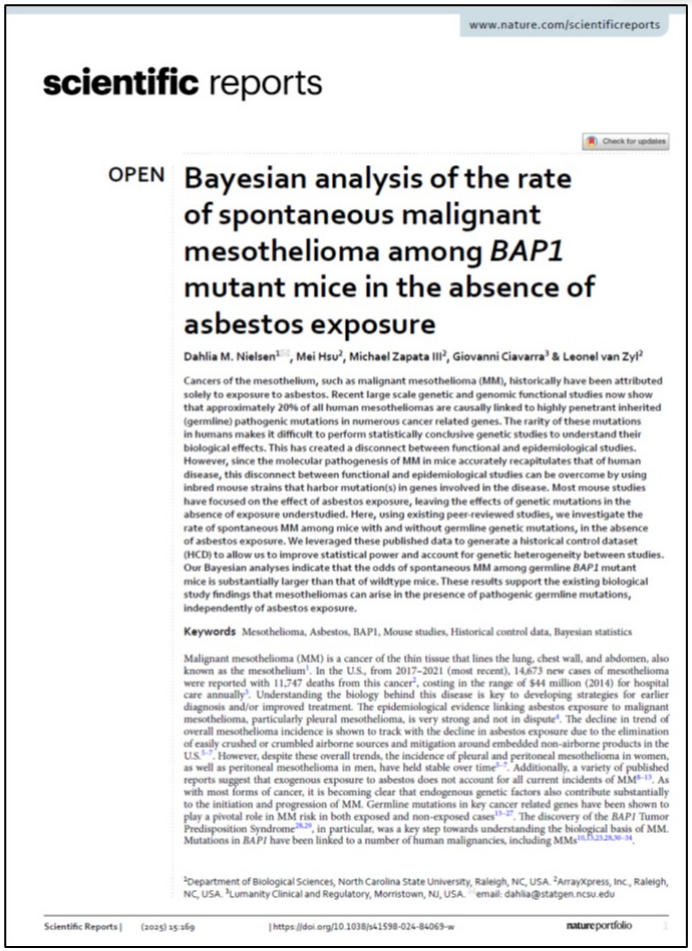


Adapted from Kadariya *et al*., 2016

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

"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



www.nature.com/scientificreports

scientific reports

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 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.

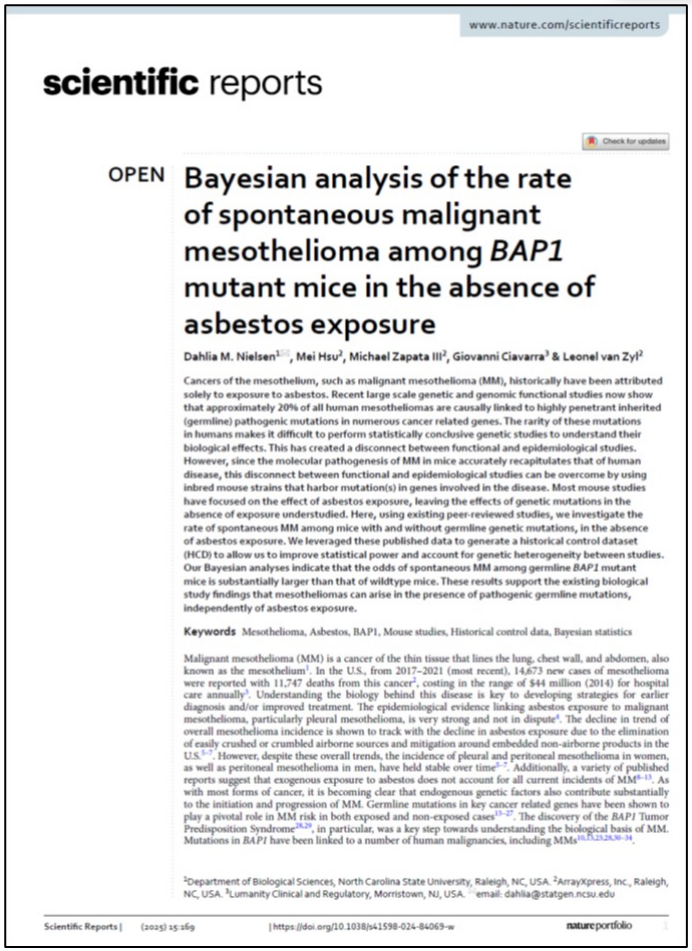
Keywords Mesothelioma, Asbestos, BAP1, Mouse studies, Historical control data, Bayesian statistics

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[8–9]. 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].

[1]Department of Biological Sciences, North Carolina State University, Raleigh, NC, USA. [2]ArrayXpress, Inc., Raleigh, NC, USA. [3]Lumanity Clinical and Regulatory, Morristown, NJ, USA. ✉email: dahlia@statgen.ncsu.edu

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]."** Nielsen *et al*., 2025



www.nature.com/scientificreports

scientific reports

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 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.

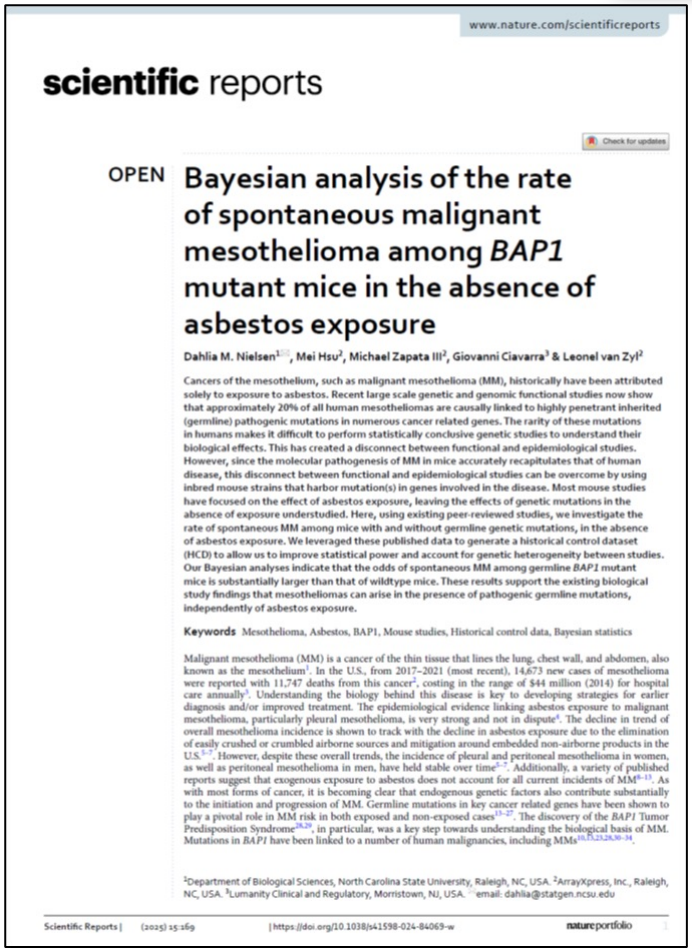
Keywords Mesothelioma, Asbestos, BAP1, Mouse studies, Historical control data, Bayesian statistics

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[8–7]. 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[10,15,23,28,30–34].

[1]Department of Biological Sciences, North Carolina State University, Raleigh, NC, USA. [2]ArrayXpress, Inc., Raleigh, NC, USA. [3]Lumanity Clinical and Regulatory, Morristown, NJ, USA. ✉email: dahlia@statgen.ncsu.edu

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[16,22,26,31,59]. Some studies estimate MM rates even as high as 36%[27]."** Nielsen *et al*., 2025
- **"Germline null mutations in several highly penetrant tumor suppressor genes, such as *BAP1* and *TP53*, demonstrate ~100% cancer penetrance[30–32,80], underscoring that some highly penetrant mutations are themselves sufficient to cause cancer[81]. Pathogenic germline null mutations in the *BAP1* gene have been empirically shown to cause MM and other cancers without exogenous exposures[5–8,23,24,26,31,32,36,37,80,82,83]."** Nielsen *et al*., 2025



www.nature.com/scientificreports

scientific reports

OPEN

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

Dahlia M. Nielsen, Mei Hsu, Michael Zapata III, Giovanni Ciavarra & Leonel van Zyl

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. In the U.S., from 2017–2021 (most recent), 14,673 new cases of mesothelioma were reported with 11,747 deaths from this cancer, costing in the range of $44 million (2014) for hospital care annually. 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. 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. 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. Additionally, a variety of published reports suggest that exogenous exposure to asbestos does not account for all current incidents of MM. 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. The discovery of the *BAP1* Tumor Predisposition Syndrome, 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.

Department of Biological Sciences, North Carolina State University, Raleigh, NC, USA. ArrayXpress, Inc., Raleigh, NC, USA. Lumanity Clinical and Regulatory, Morristown, NJ, USA. email: dahlia@statgen.ncsu.edu

Scientific Reports | (2025) 15:169 | https://doi.org/10.1038/s41598-024-84069-w nature portfolio

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

CANCER

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

Meng He[1], Mira S. Chaurushiya[1+], Joshua D. Webster[2], Sarah Kummerfeld[3†], Rohit Reja[3], Subhra Chaudhuri[4], Ying-Jiun Chen[4], Zora Modrusan[4], Benjamin Haley[4], Debra L. Dugger[1], Jeffrey Eastham-Anderson[2], Shari Lau[2], Anwesha Dey[3], Roger Caothien[3], Merone Roose-Girma[4], Kim Newton[1‡], Vishva M. Dixit[1‡]

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 prosurvival genes *Bcl2* and *Mcl1*. In contrast, BAP1 loss in melanocytes had little impact on expression of prosurvival 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 commutated with *BRAF* in melanoma (*6*), *GNAQ* or *GNA11* in uveal melanoma (*7*), and *CDKN2A* or *NF2* 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 thoracic 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^{T2} Bap1$^{fC91A/fC91A}$*) that express catalytically inactive BAP1 Cys^{91}→Ala (C91A) instead of wild-type BAP1 after tamoxifen treatment (fig. S1A). *Bap1$^{C91A/-}$* embryonic stem (ES) cells derived from these mice expressed amounts of BAP1 C91A comparable to those of wild-type BAP1 in *Bap1$^{+/-}$* control ES cells (fig. S1B). Tamoxifen-induced expression of BAP1 C91A in adult mice caused splenomegaly, leukocytosis, neutrophilia, 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.

Compared with their wild-type or *Bap1$^{+/-}$* counterparts, *Bap1$^{C91A/-}$* and *Bap1$^{-/-}$* liver and pancreas showed increased immunolabeling of cleaved caspase-3, a hallmark of apoptosis (Fig. 1, A and B, and fig. S1, I and J). These data prompted us to explore whether BAP1 is critical for the survival of other cell types. BAP1 loss or inactivation also killed cultured ES cells (Fig. 1C and fig. S2A), primary keratinocytes (fig. S2B), and E1A-immortalized mouse embryo fibroblasts (MEFs) (fig. S2C). The cells appeared to die by apoptosis because caspase-3 and its substrate PARP [poly(ADP-ribose) polymerase] were cleaved (fig. S2D), and cell death was inhibited by the pan-caspase inhibitor emricasan (fig. S2E). We used a genome-wide CRISPR-Cas9 screen (fig. S2F and table S1) to identify *Rnf2* as a gene promoting the death of *Bap1$^{-/-}$* MEFs (Fig. 1D). RNF2 is one of only two E3 ubiquitin ligases that modify histone H2A with monoubiquitin at residue Lys^{119} (H2AK119Ub) to suppress gene transcription (*17*, *18*), whereas BAP1 removes H2AK119Ub (*19*). Thus, increased RNF2-dependent ubiquitination of histone H2A after BAP1 inactivation may promote cell death. RNF2 deletion improved the viability of *Bap1$^{-/-}$* ES cells, whereas deletion of RING1, the other E3 that generates H2AK119Ub, provided little benefit (fig. S2, G to J). However, the combined loss of RNF2 and RING1 improved survival of *Bap1$^{-/-}$* ES cells more than RNF2 loss alone (fig. S2, I and J). Therefore, RNF2 and RING1 both appear to contribute to the death of *Bap1$^{-/-}$* cells.

If BAP1 deubiquitinates H2AK119Ub made by RNF2, then the enzymatic activity of RNF2 should be required for the death of *Bap1$^{-/-}$* cells. Indeed, BAP1 deficiency killed *Ring1$^{-/-}$Rnf2$^{-/-}$* ES cells reconstituted with wild-type RNF2 but not those expressing catalytically inactive RNF2 153S (I, Ile; S, Ser) (*20*) (Fig. 2, A and B). To test whether RNF2 might suppress genes required for cell survival in *Bap1$^{-/-}$* cells, we used RNA sequencing (RNA-seq) to compare *Bap1$^{+/+}$*, *Bap1$^{+/-}$*, and *Bap1$^{-/-}$*

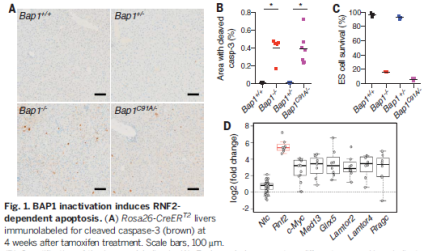
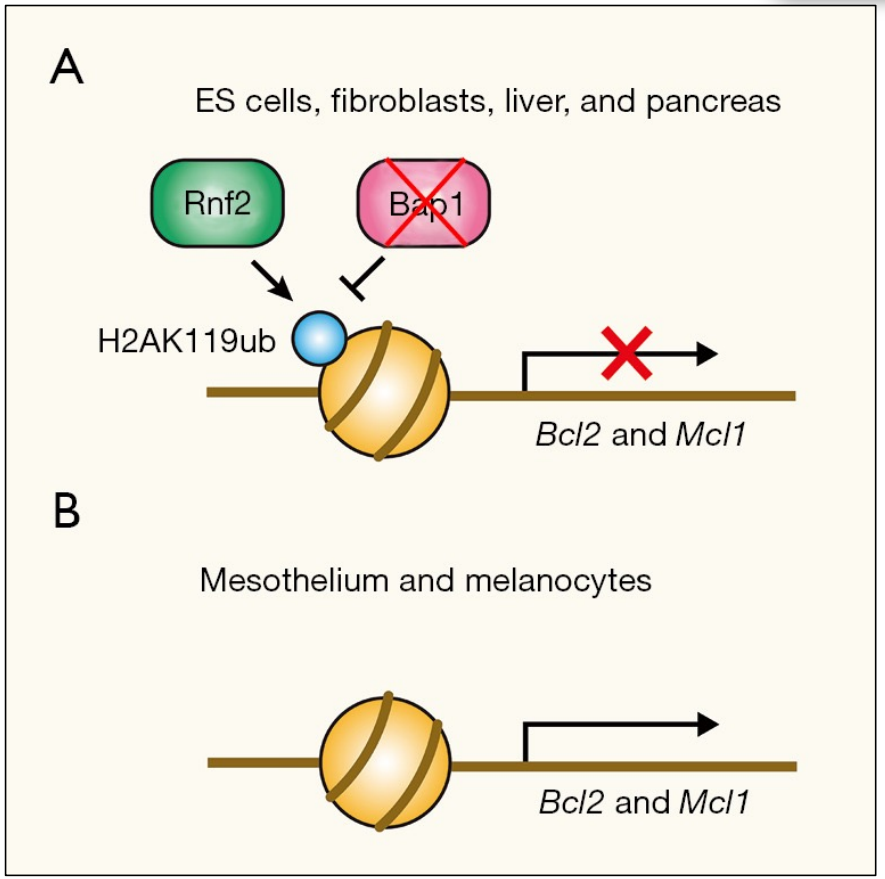


Fig. 1. BAP1 inactivation induces RNF2-dependent apoptosis. (**A**) *Rosa26-CreER^{T2}* livers immunolabeled for cleaved caspase-3 (brown) at 4 weeks after tamoxifen treatment. Scale bars, 100 μm. (**B**) Quantification of the immunolabeling in (A). Each symbol represents a different mouse. Lines indicate 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^{T2}* ES cells at 6 days after beginning 4-OHT treatment. Each symbol represents a technical replicate. Lines indicate the mean. (**D**) Graph indicates guide RNA (gRNA) enrichment in MEFs surviving *Bap1* deletion. Each dot represents a distinct gRNA. Ntc, nontargeting control.

[1]Department of Physiological Chemistry, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA. [2]Department of Pathology, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA. [3]Department of Bioinformatics, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA. [4]Department of Molecular Biology, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA. [5]Department of Discovery Oncology, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA.
*Present address: SAM Ventures, 501 2nd Street #350, San Francisco, CA 94107, USA. †Present address: Kinghorn Centre for Clinical Genomics, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, NSW 2010, Australia.
‡Corresponding author. Email: dixit@gene.com (V.M.D.); knewton@gene.com (K.N.)

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



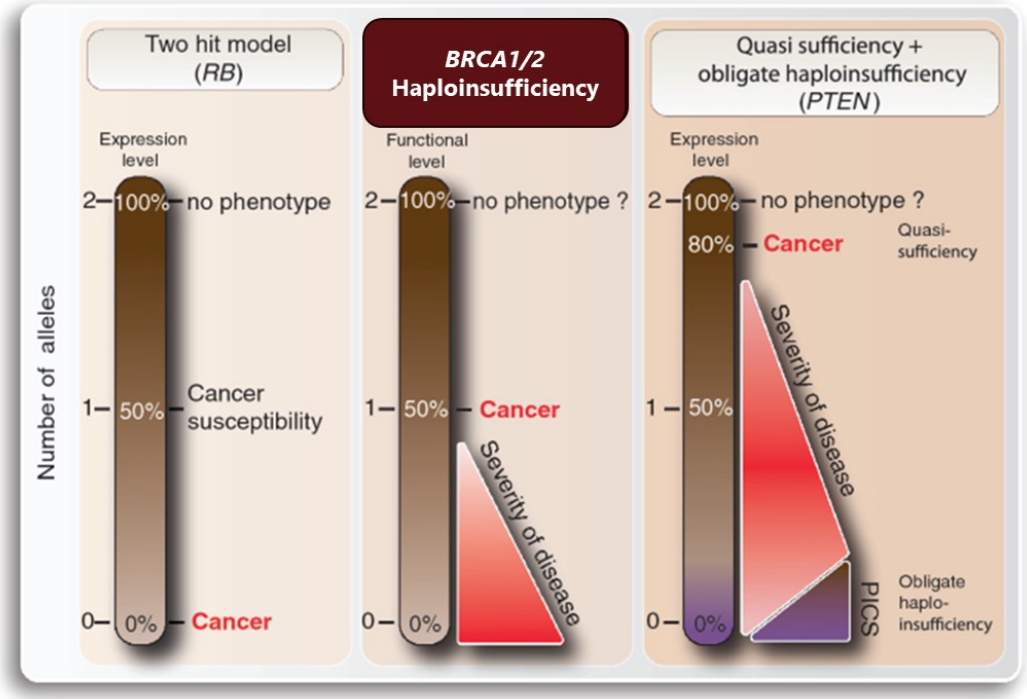
Mono-Allelic (Heterozygous) *BRCA1/2* Inactivation Suffices for Carcinogenesis

"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

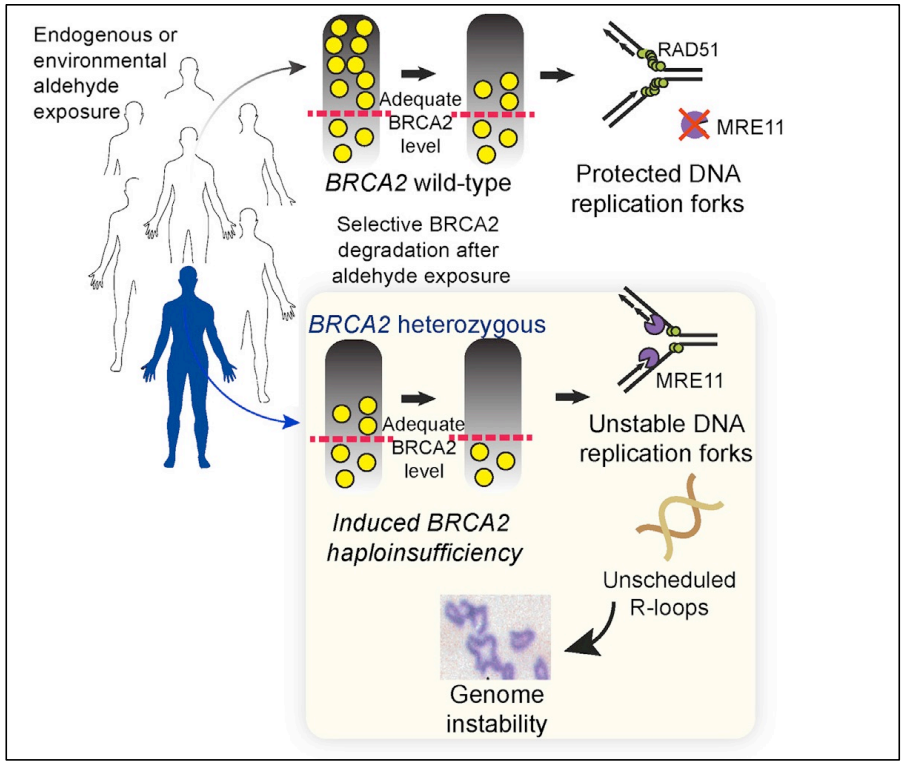


A brown gradient represents a continuum of expression related to the number of alleles present (black numbering). **Left, the two-hit paradigm as exemplified by the tumor suppressor, RB. Loss of one allele induces cancer susceptibility; loss of two alleles induces cancer**. Middle, classical haploinsufficiency 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

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

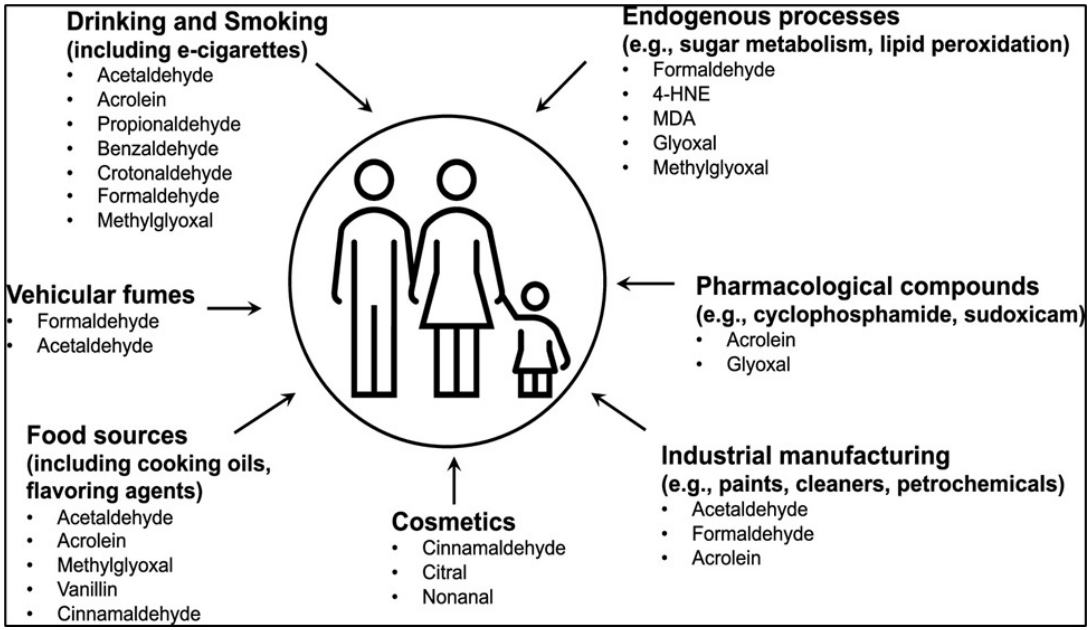
"…endogenous metabolites like aldehydes… through cellular metabolism in different tissues, are able to trigger DNA damage, induce BRCA2 haploinsufficiency accompanied by the outgrowth of genomically unstable progeny (Tan et al., 2017), or even cell death (Tacconi et al., 2017) in BRCA-mutant cells. Aldehyde 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

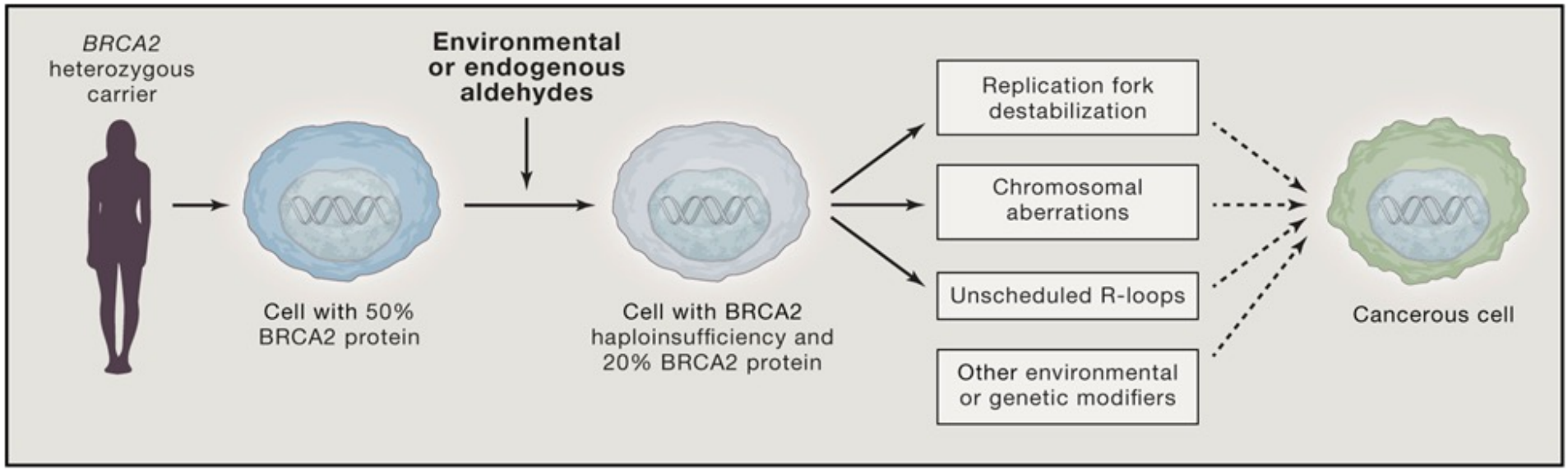
Aldehydes are widespread in the environment, with multiple environmental (external) sources such as cigarette smoke, alcohol consumption, food and beverages, industrial effluents, and additives.

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 abducts, 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).

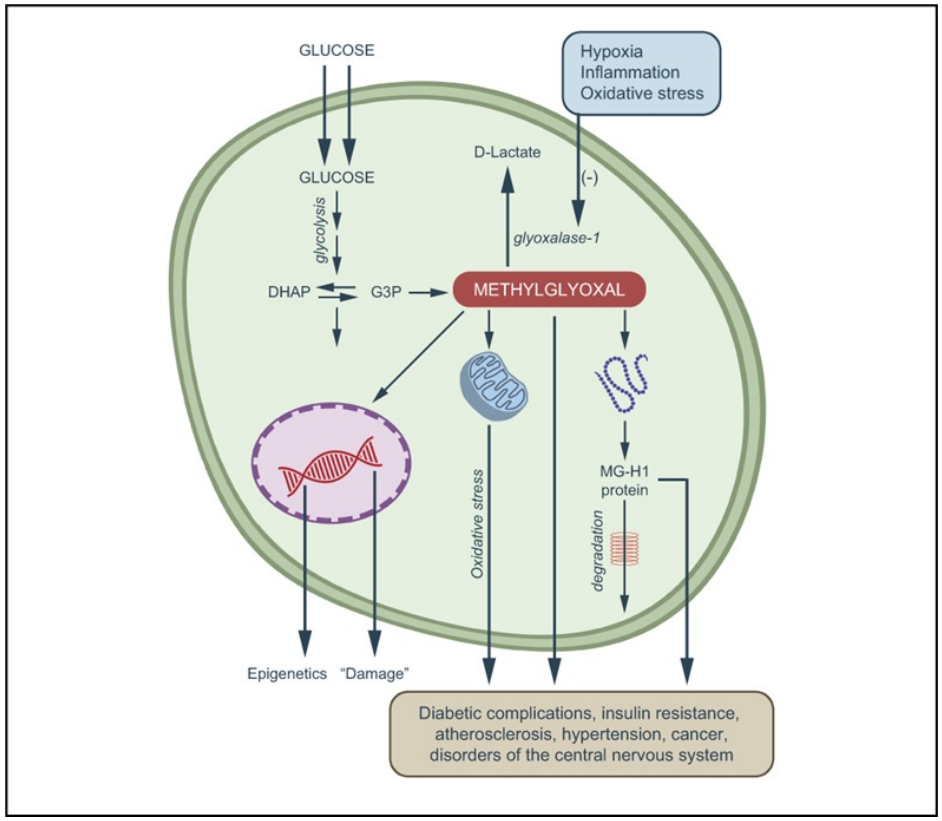
As a result, aldehyde exposure, especially from environmental (exogenous) and endogenous (byproduct of metabolism) sources, poses a high risk to human health (Seitz and Stickel, 2010; Friedenson, 2011; Inci *et al*., 2013; Parmar and D'Andrea, 2017; Reingruber and Pontel, 2018; Garaycoechea *et al*.,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



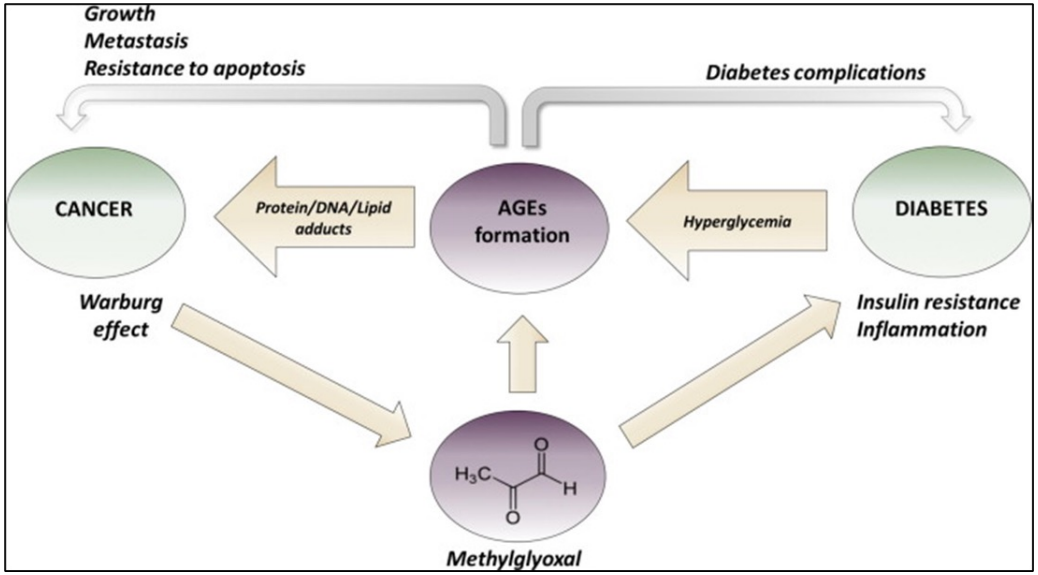
Adapted from Schalkwijk and Stehouwer, 2020

"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

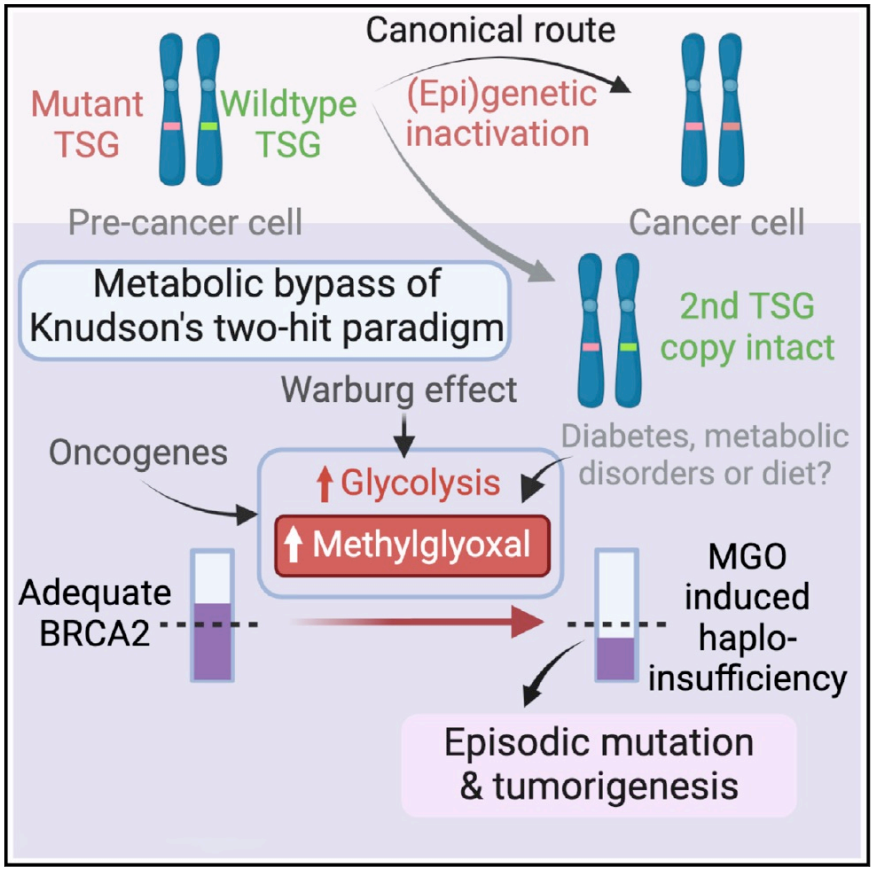


Adapted from Bellier *et al*., 2019

"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"



Adapted from Kong *et al*., 2024

Methylglyoxal

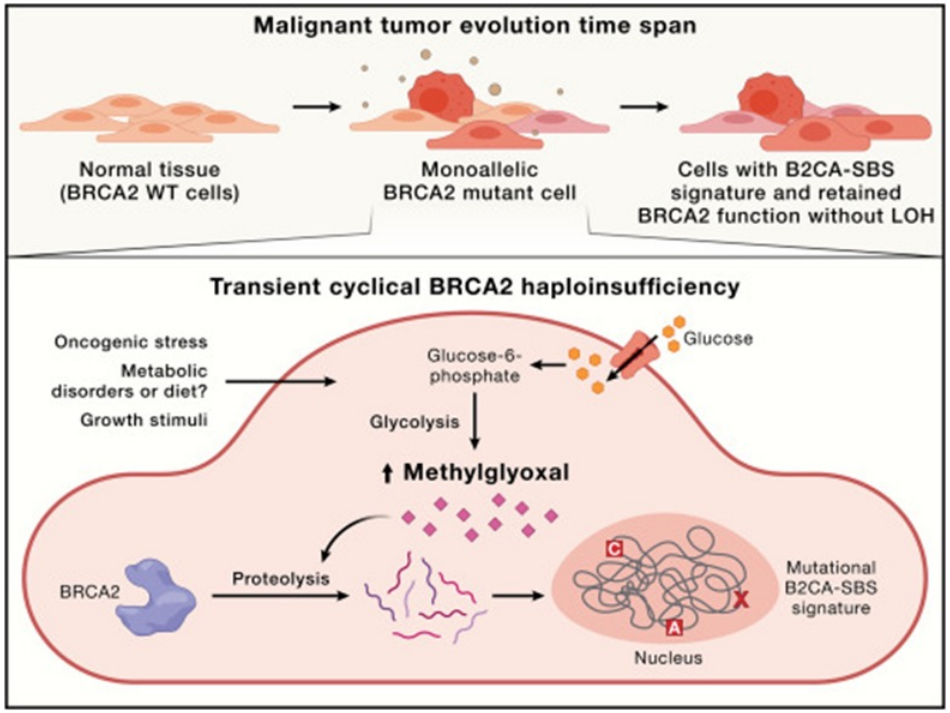
"MGO transiently inactivates BRCA2 function, bypassing Knudson's ''two-hit'' requirement"

"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

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

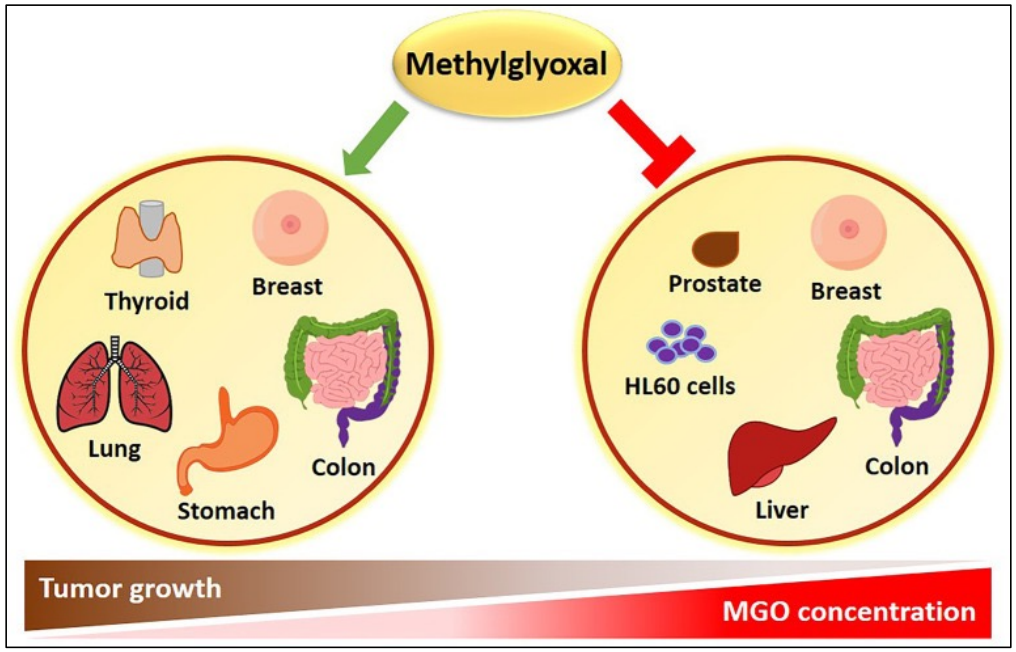


Adapted from Jiang, 2024

"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-$_K$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

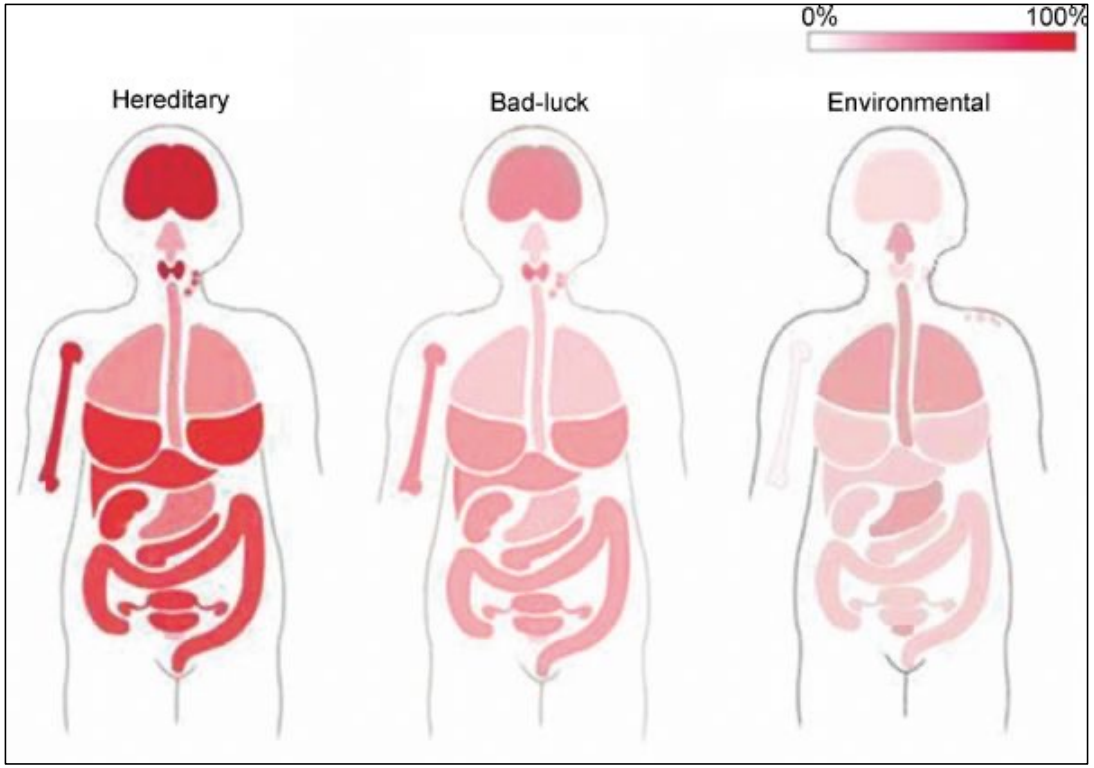
"In this model, the risk of carcinogenesis among mutation carriers may depend not only on the nature of their germline BRCA2 mutation and its susceptibility to aldehyde-induced haploinsufficiency but also upon other genetic and environmental factors that determine exposure to aldehydes. Because BRCA2 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**

ToxicoGenomica

Current Status of Acquiring and Utilizing Genomic Data

Michael Zapata III
mz3@arrayxpress.com
919.696.7814

ARRAYXPRESS
Your Contract Research Partner for Next Generation Genomics

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 outdate “panel”
  - A subtraction methodology that is residual from an outdate 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

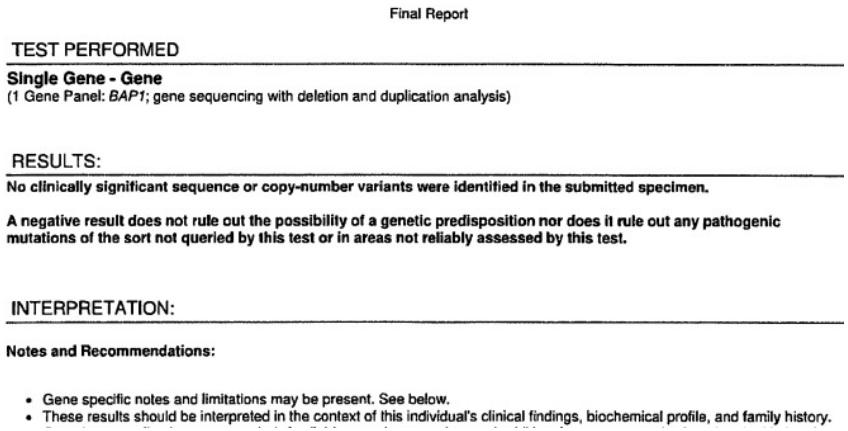
- The data itself can be raw sequencing files, output files, spreadsheets, or other proprietary formats
- For whole genome sequencing the raw data can be in the terabytes and take many hours to download for processing, then it is just raw data
- Exome data is in the gigabytes because it is a fraction 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



Final Report

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.

- 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

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, 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

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

Caveats and Copyright

- **Presentation slides composed and provided for educational and informative purposes, with the caveat that they are condensed and imperfect methods for communicating sometimes complex information**
- **The individual and collective slides are not intended to cover all factors that may be relevant to a particular situation, and should be considered in context**
-

ToxicoGenomica

ArrayXpress
mz3@arrayxpress.com
919.696.7814

LSP Group
khartley@lspgrp.com
312.802.4471