Genetic Testing for Gynecological Cancers: Inherited vs Tumor Testing

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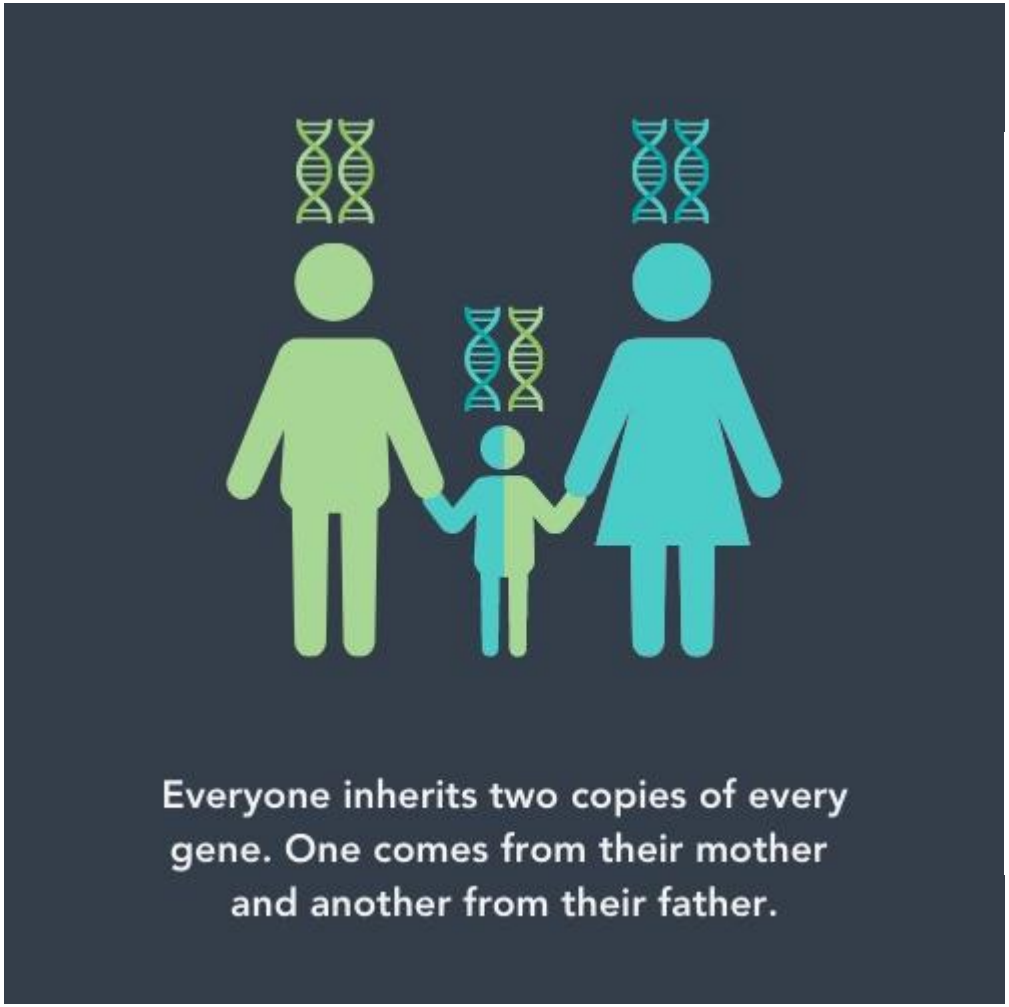
Disclosures

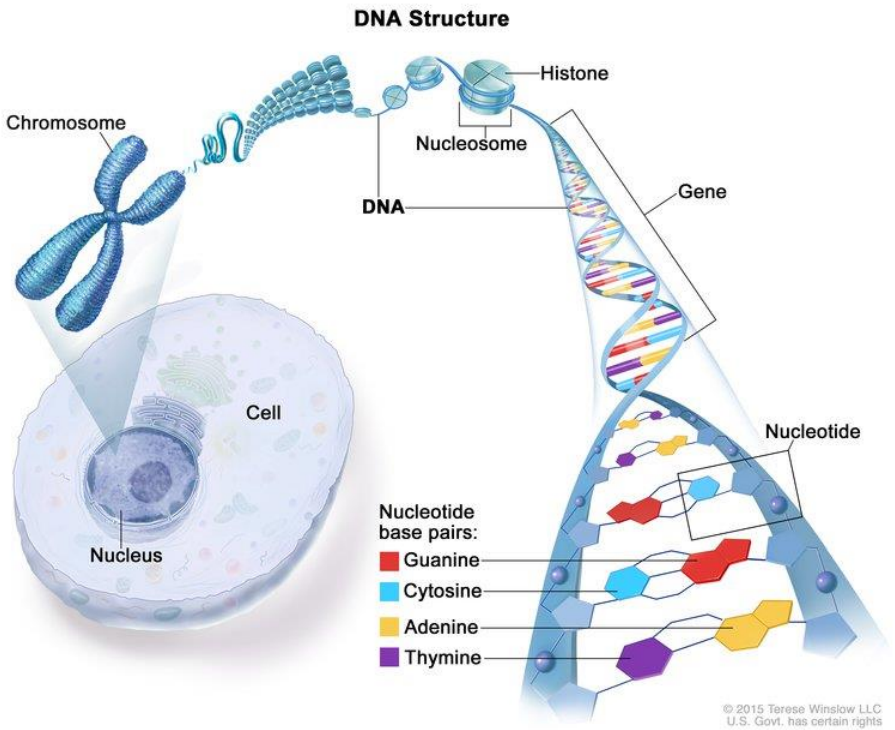
- No conflicts of interest to disclose

Outline

- Cancer – a condition of genetic mutations
- Knowing about these mutations is important
- Testing for genetic mutations in a tumor
- Genetic mutations that are inherited
- Testing for inherited genetic mutations
- How this information is useful together
- Q&A

Genetics Background





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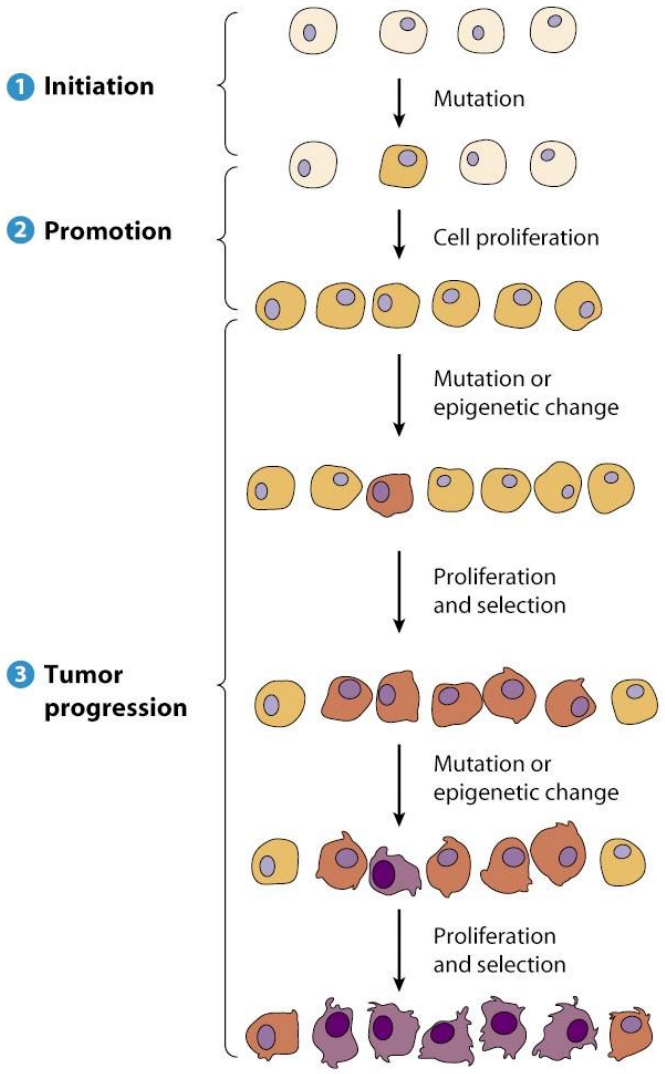
What is Cancer?



- Disease in which cells divide without control
  - Mass or tumor with ability to invade other tissues
- Results from malfunctioning regulation processes
  - Cells have innate ability to control their own replication through DNA
    - "Oncogenes" or "tumor suppressor genes"
- Genetic mutation is what disturbs regulation
- Cancer is the result of genetic mutations

Why know about tumor mutations?

- Explore what is “driving” a tumor
  - Mechanisms of growth and how mutations interact with one another
  - Target for treatment or therapies, can we exploit genetic mutations?
  - Help in tissue identification
  - More complete picture about an individual case

Pathogenic or Likely Pathogenic Somatic Alterations	
Variant	**CLASSIFICATION**
ARID1A p.G276fs	Pathogenic
ESR1 p.Y537N	Pathogenic
JAK1 p.K860fs	Pathogenic
KRAS p.G12V	Pathogenic
PIK3CA p.E545G	Pathogenic
PTEN p.S229fs	Pathogenic
NF1 c.7970+2T>A	Likely Pathogenic
SETD2 c.S1366fs	Likely Pathogenic
ZFHX3 p.G699fs	Likely Pathogenic

Ways to do Tumor Genetic Testing

- Solid tumor biopsy or "tumor profiling"

FOUNDATIONONE® CDx

oncotype DX®
Breast Recurrence Score

CARIS® LIFE SCIENCES

AGENDIA® MAMMAPRINT
Learn the likelihood of a patient's breast cancer recurring.
A RISK OF RECURRENCE TEST

AGENDIA® BLUEPRINT
Determine the underlying genomics driving the growth of a tumor.
A MOLECULAR SUBTYPING TEST

- Liquid biopsy (blood or urine)

FOUNDATIONONE® LIQUID CDx

GUARDANT360™

Case Example

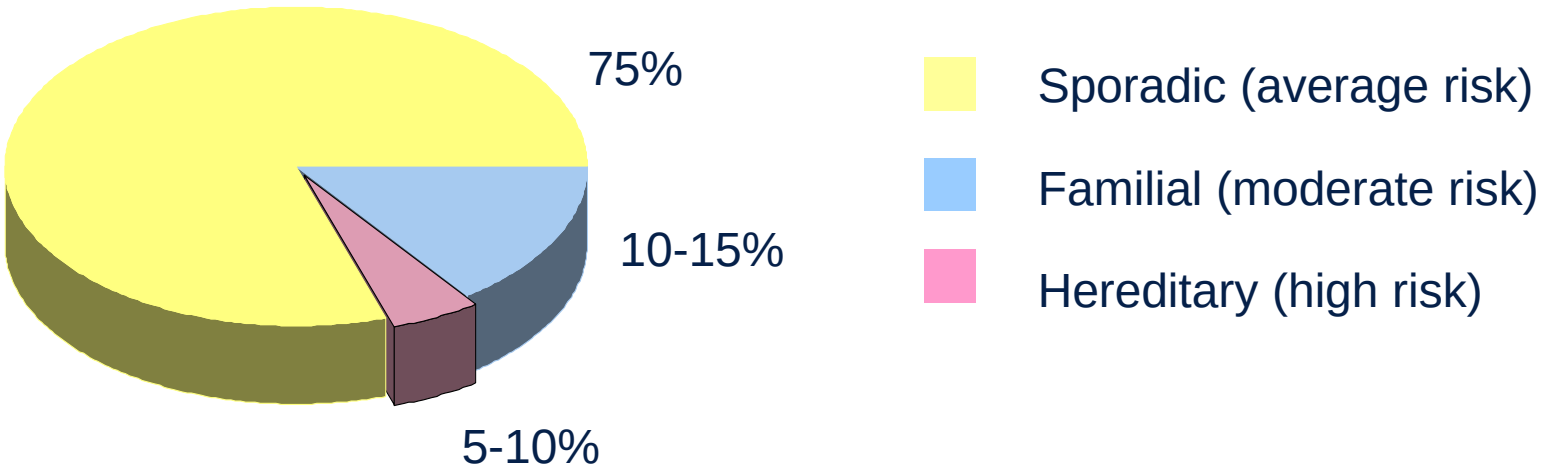
- Women in 50s presented with signs of advanced pancreatic cancer, pathologists confirm
- Treated at UCSF, new ovarian mass found as cancer is progressing
- Biopsy of the mass identified ovarian cancer instead due to genetic mutations
  - *KRAS*
- Chemo attempted with ovarian cancer-specific medication
- Completed reversed symptoms, and able to achieve remission with chemotherapy and surgery

- Power of tumor genetic testing application

Inherited Genetic Mutations

- Most cancerous genetic mutations are acquired during one's lifetime
- Some rare individuals have a genetic mutation present from birth, in every cell of the body
  - Typically inherited from one parent (in original egg and/or sperm)
- Easier to develop cancer during lifetime
  - "Predisposition" to cancer
- Some cancer presentation is more likely to involve an inherited cancer risk factor than others
- Shared cancer risk between family members

Inherited Mutations are (somewhat) rare



- Ovarian cancer – 20% (Walsh et al., 2011)
- Endometrial (uterine) cancer – 5-10% (Lu et al., 2007)
- Uterine sarcoma – unknown
- Cervical cancer – minimal inherited factors

Why know about inherited mutations?

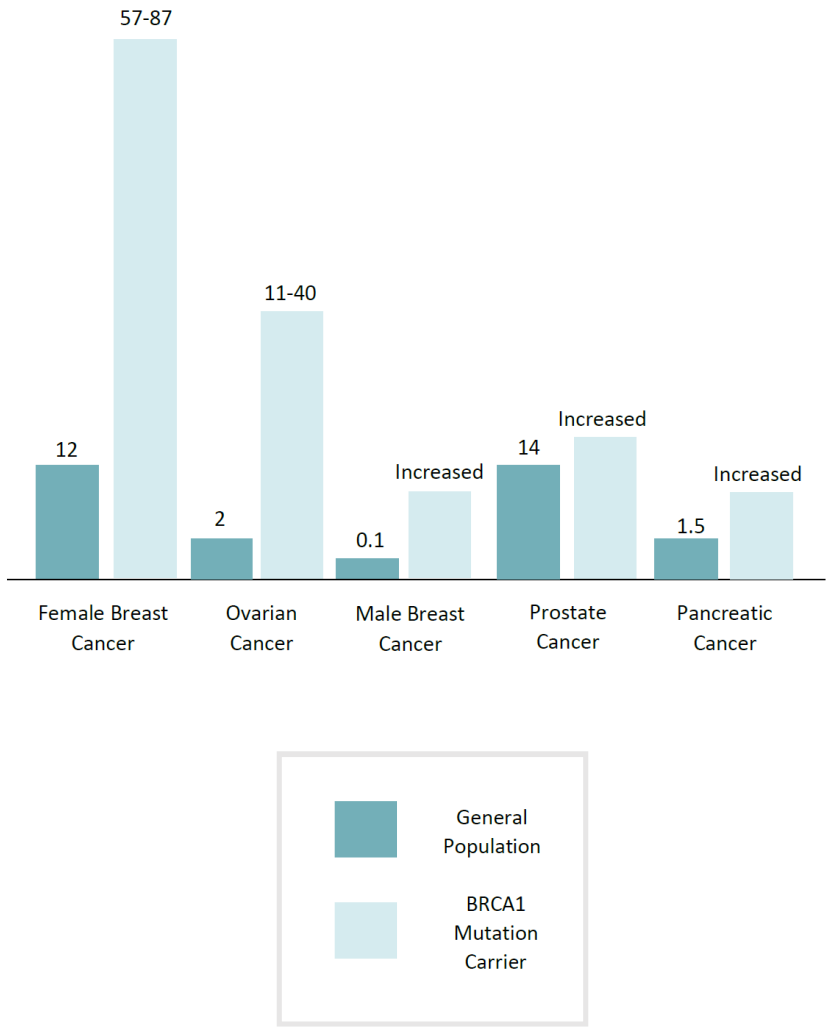
- More accurately prediction of lifetime cancer risks
- Similarities between an individual's cells and their cancer cells
- Other cancer risk profiles
- Specific tumor pathologies more likely, used to drive care and treatment
  - Impact on surgical decisions
  - Impact on treatment/medication
- Indicated genetic testing for family members

Germline genetic testing options

- Performed on healthy “normal” tissue
  - Blood sample
  - Liquid saliva or cheek cells (buccal)
- White blood cell content
- Can tailor amount of information, number of genes
- Sequencing and deletion/duplication

Case Example

- Patient presents with ovarian cancer
- *BRCA1* inherited gene mutation
- Family history of prostate cancer
- Could now:
  - Benefit from PARP inhibitor
  - Qualify for clinical trials
  - Be aware of other cancer risks for herself
- Risk for family members, regardless of sex



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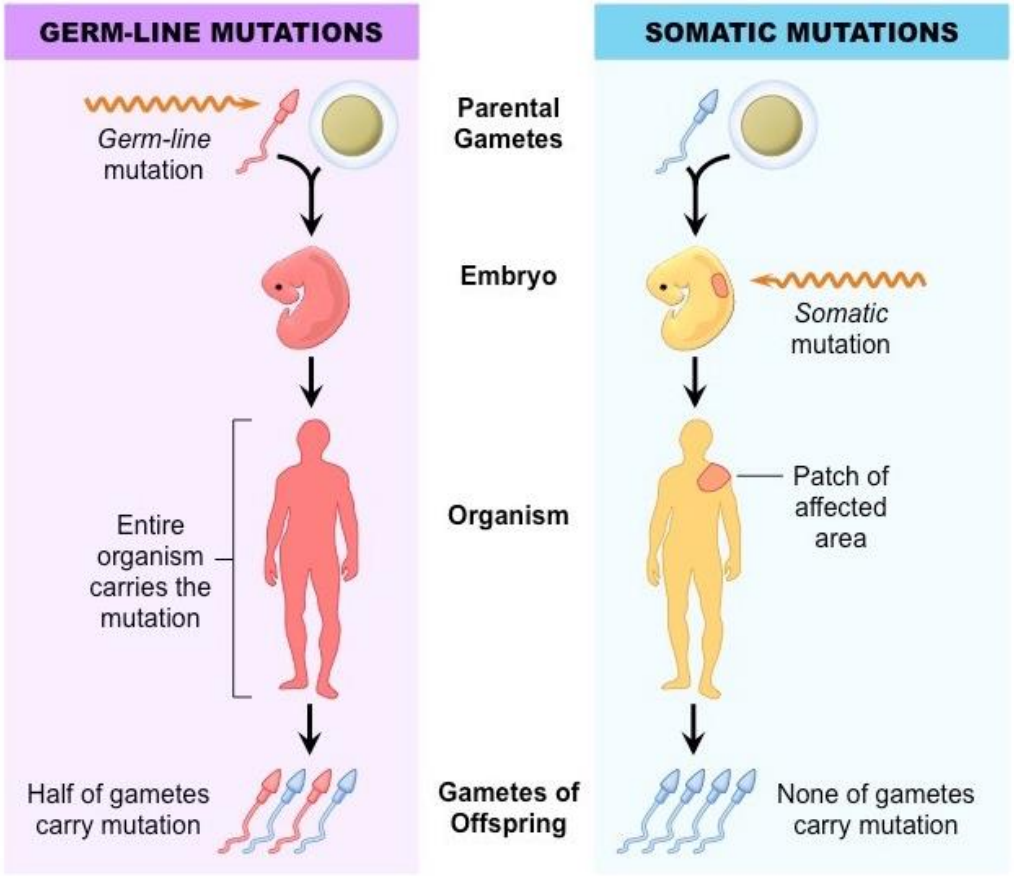
What does each kind of test tell us about the other?

- Not much…
  - Liquid biopsy testing has NO information on inherited genes
  - Solid tumor testing MAY contain some other information
  - Inherited genetic testing DOES NOT report on tumor mutations



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Combined testing ("Paired") is also an option

UCSF500 Gene Panel Final Report

CCGL No: [redacted]
Date: [redacted]

Patient: [redacted]
MRN: [redacted]
DOB: [redacted]
Sex: [redacted]
Ordering Provider(s): [redacted]
Surgical Pathologist: [redacted]
Resident/Fellow: [redacted]
Electronically Signed-Out by: [redacted]

Tumor
Source: [redacted] Fallopian tube, Solid Tissue [redacted]
Diagnosis: Several small foci of high grade serous carcinoma
Collected: [redacted]

Normal
Source: Peripheral Blood [redacted]
Collected: [redacted]

Pathogenic or Likely Pathogenic SOMATIC ALTERATIONS

VARIANT	TRANSCRIPT ID	CLASSIFICATION	READS	MUTANT ALLELE FREQUENCY
BRCA1 loss of wild-type allele	N/A	Pathogenic	N/A	N/A
TERT c.-124C>T	NM_198253.2	Pathogenic	865	38%
TP53 p.I195T	NM_000546.5	Pathogenic	513	61%
PBRM1 c.528+2T>G	NM_018313.4	Likely Pathogenic	595	42%
SF3B1 p.G742D	NM_012433.2	Possibly Pathogenic	503	29%

'Reads' indicates the number of unique DNA molecules sequenced. 'Mutant Allele Frequency' indicates the percentage of the reads with the respective 'Variant' and is affected by the degree of normal cell contamination of the sample and whether the variant is fully clonal or subclonal. 'Pathogenic' and 'Likely Pathogenic' classifications are based on CCGL molecular pathologist/geneticist interpretation of data from somatic and germline databases and published literature. Variants classified as 'Possibly Pathogenic' have unknown significance but occur in genes or molecular pathways known to be recurrently altered in the tumor type.

Pathogenic or Likely Pathogenic ALTERATIONS IN THE NORMAL SAMPLE*

VARIANT	TRANSCRIPT ID	CLASSIFICATION	READS (Normal/Tumor)	MUTANT ALLELE FREQUENCY (Normal/Tumor)
BRCA1 p.P1192fs (c.3575delC, p.Pro1192fs)	NM_007294.3	Pathogenic	729/609	53%/92%

*Alterations in the normal sample are reported for cancer-related genes if classified as pathogenic or likely pathogenic in ClinVar and confirmed by a CCGL molecular pathologist/geneticist. For variants not classified in ClinVar, truncating or splice-site variants in well-established tumor suppressor genes are reported if present in <1% of 1000g or esp6500 datasets. Alterations in the normal samples are limited to single nucleotide variants and small indels in gene coding regions. Carrier status is not reported for variants not strongly related to cancer.

45 of 1036 tested microsatellites (4.34%) were found to be unstable.
Assessment of microsatellite instability (MSI) by percentage of unstable sites:
<20%: MSI absent (MSS) | 20-30%: MSI equivocal | >30%: MSI present (MSI-High)

INTERPRETATION

Identified pathogenic/likely pathogenic somatic mutations include a recurrent missense mutation in TP53 (p.I195T) with loss of the remaining wild-type allele, a hotspot TERT promoter mutation (c.-124C>T), and a splice site mutation in PBRM1 (c.528+2T>G). A hotspot SF3B1 mutation (p.G742D) was also identified. Copy number analysis revealed numerous chromosomal gains and losses without pathogenic focal amplifications or deep deletions.

A heterozygous truncating germline BRCA1 mutation (c.3575delC, p.P1192fs) was identified in the peripheral blood sample, which shows loss of heterozygosity in the tumor sample, consistent with a driver function in this high grade serous carcinoma.

Ovarian high-grade serous carcinomas universally harbor TP53 mutations and are frequently associated with germline BRCA1 mutations (1, 2). Among ovarian carcinomas, TERT promoter mutations are most characteristically associated with clear cell carcinomas and have only very rarely been reported in serous carcinomas (3). PBRM1 encodes a component of the SWI/SNF chromatin remodeling pathway. SF3B1 encodes a spliceosomal component that is frequently mutated in hematologic neoplasms and relatively infrequently mutated in solid tumors.

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Summary

Inherited Genetic Testing ("Germline")

- Identify inherited risk factors for cancers
- Blood or saliva sample
- Smaller number of genes
- Impact on current cancer treatment
- Impact for family members

- Tumor Genetic Testing ("Somatic")
- Identify mutations that drive growth of a cancer
- Tumor tissue or blood sample testing for circulating tumor DNA
- Larger number of genes
- Impact on current cancer treatment

Thanks to the Cancer Genetics and Prevention Program (CGPP)

- Director Amie Blanco
- Supervisor Julie Mak
- Team of 17 Genetic Counselor specializing in Cancer Risk
- Specialties in Gyn/Onc as well as breast cancer, colon cancer, childhood cancers, etc.
- Hereditary Cancer Clinic

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Extra Slides

Oncogenes (Proto-oncogenes) vs Tumor Suppressor Genes

- Proto-oncogenes become oncogenes with mutation
- Altered products that increase/active cell division
  - Activation causes cancer
- Mostly occur in tumor tissue (rarely in inherited DNA)
- *Ras* gene, *HER-2*, *EGFR*

- Protective genes that control cell growth
- Mutations cause stops to normal cell cycle, leading to tumor development
  - Inactivation causes cancer
- Can occur in tumor tissue or inherited DNA (germline)
- *BRCA1* and *BRCA2*, *APC*, MMR gene mutations

"Two-Hit" Hypothesis of Hereditary Cancer

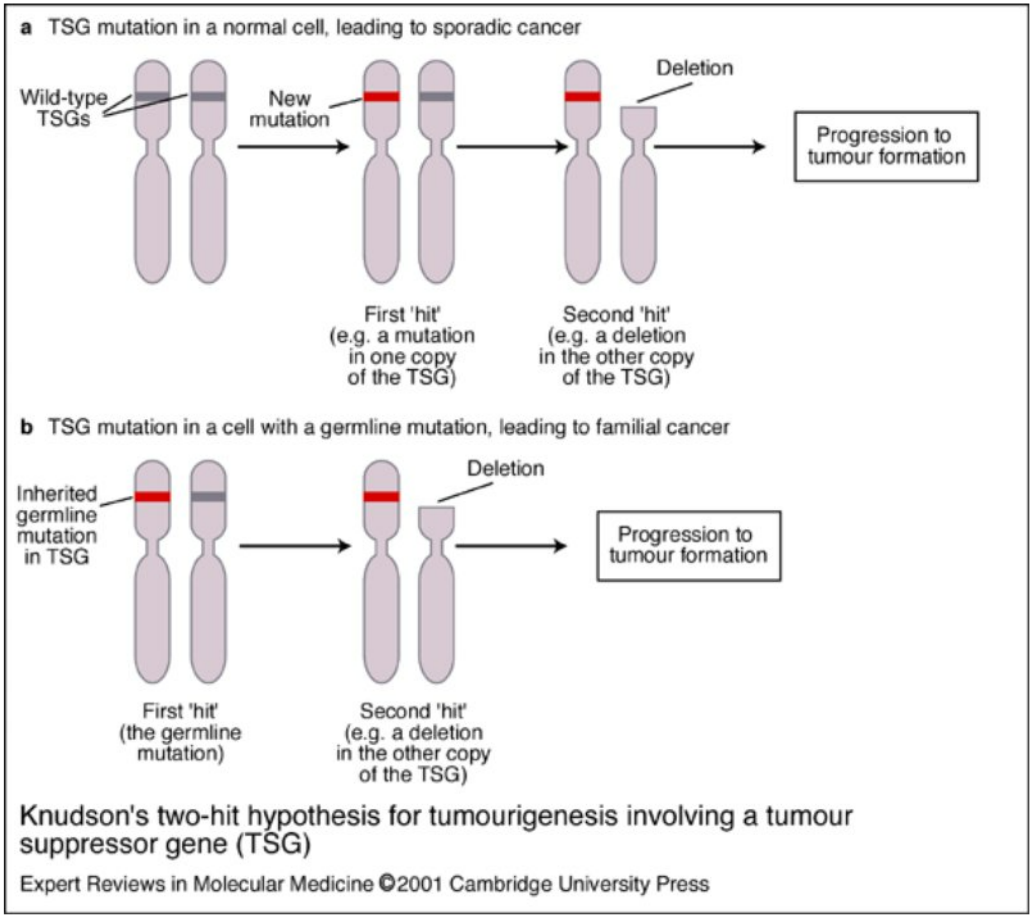

Figure 1. Illustration of Knudson's two-hit hypothesis of tumor suppressor inactivation in familial (hereditable) cancer. 10 Knudson, A.G. (1971) Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A 68, 820-823,PubMed.

Reference

1. Knudson AG Jr., 1971, *Mutation and cancer: statistical study of retinoblastoma.* Proc Natl Acad Sci U S A. Apr;68(4):820-3.

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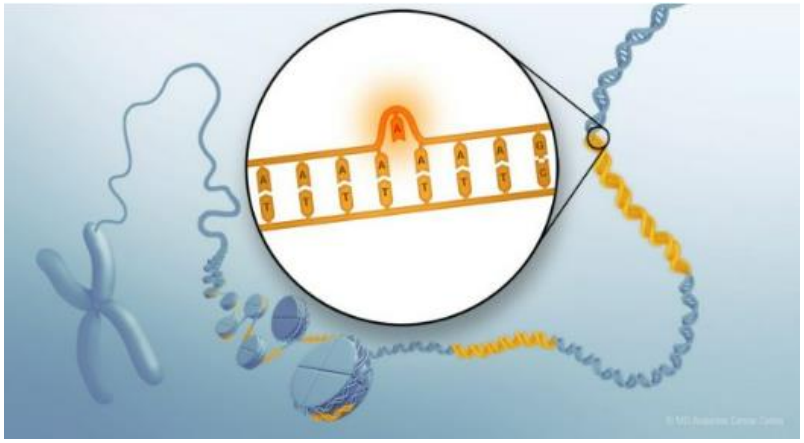
Microsatellites

- Short repetitive sequences in the DNA
- Biomarker for DNA stability
  - Microsatellite instability (MSI)
- Functioning genes keep these microsatellite regions in order through DNA mismatch repair (MMR)



- “Mismatch repair deficiency” = “MSI-high” = “MMR absent”
- MMR gene mutations can be inherited
  - *MLH1, PMS2, MSH2, MSH6, EPCAM*

INVITAE

INVITAE DIAGNOSTIC TESTING RESULTS

Patient name: John Doe	**Sample type:** Blood	**Report date:**
DOB:	**Sample collection date:**	**Invitae #:**
Sex:	**Sample accession date:**	**Clinical team:**
MRN:		

Reason for testing
Diagnostic test for a personal and family history of disease

Test performed
Sequence analysis and deletion/duplication testing of the 83 genes listed in the results section below.

- Invitae Multi-Cancer Panel

RESULT: POSITIVE

One Pathogenic variant identified in BRCA2. BRCA2 is associated with autosomal dominant hereditary breast and ovarian cancer syndrome and autosomal recessive Fanconi anemia.
Additional Variant(s) of Uncertain Significance identified.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
BRCA2	c.4638del (p.Phe1546Leufs*22)	heterozygous	PATHOGENIC
PALB2	c.2482T>C (p.Cys828Arg)	heterozygous	Uncertain Significance

About this test
This diagnostic test evaluates 83 gene(s) for variants (genetic changes) that are associated with genetic disorders. Diagnostic genetic testing, when combined with family history and other medical results, may provide information to clarify individual risk, support a clinical diagnosis, and assist with the development of a personalized treatment and management strategy.

Sample Invitae cancer panel report.

Genetic Result - Integrated BRACAnalysis®
BRCA1 and *BRCA2* Analysis

BRACAnalysis® Powered by myVision

RECEIVING HEALTHCARE PROVIDER	SPECIMEN		PATIENT	
Test HCP, MD	Specimen Type:	Blood	Name:	Pt Last Name, Pt First Name
Test Medical Center	Draw Date:	Jun 09, 2016	Date of Birth:	
123 Main St	Accession Date:	Jun 09, 2016	Patient ID:	Patient id
Testville, TX 55555	Report Date:	Jun 14, 2016	Gender:	Female
			Accession #:	07001268-BLD
			Requisition #:	7001268

RESULT: POSITIVE - CLINICALLY SIGNIFICANT MUTATION IDENTIFIED
Note: "CLINICALLY SIGNIFICANT," as defined in this report, is a genetic change that is associated with the potential to alter medical intervention.

GENE	MUTATION	INTERPRETATION
BRCA2	c.xxxxxxxxxxx Heterozygous	High Cancer Risk This patient has Hereditary Breast and Ovarian Cancer syndrome (HBOC).

DETAILS ABOUT: *BRCA2* c.xxxxxx: NM_000059.3; (aka: xxxxx)

Functional Significance: Deleterious - Abnormal Protein Production and/or Function
The heterozygous germline *BRCA2* mutation c.xxxxx is predicted to result in the premature truncation of the *BRCA2* protein at amino acid position xxxx (p.xxxxx).

Clinical Significance: High Cancer Risk
This mutation is associated with increased cancer risk and should be regarded as clinically significant.

ADDITIONAL FINDINGS: NO VARIANT(S) OF UNCERTAIN SIGNIFICANCE (VUS) IDENTIFIED

Details About Non-Clinically Significant Variants: All individuals carry DNA changes (i.e., variants), and most variants do not increase an individual's risk of cancer or other diseases. When identified, variants of uncertain significance (VUS) are reported. Likely benign variants (Favor Polymorphisms) and benign variants (Polymorphisms) are not reported and available data indicate that these variants most likely do not cause increased cancer risk. Present evidence does not suggest that non-clinically significant variant findings be used to modify patient medical management beyond what is indicated by the personal and family history and any other clinically significant findings.

Variant Classification: Myriad's myVision™ Variant Classification Program performs ongoing evaluations of variant classifications. In certain cases, healthcare providers may be contacted for more clinical information or to arrange family testing to aid in variant classification. When new evidence about a variant is identified and determined to result in clinical significance and management change, that information will automatically be made available to the healthcare provider through an amended report.

ADDITIONAL INFORMATION

GENES ANALYZED

Unless otherwise noted sequencing and large rearrangement analyses were performed on the following genes:

BRCA1, BRCA2

Indication for Testing: It is our understanding that this individual was identified for testing due to a personal or family history suggestive of a hereditary predisposition for cancer.

Associated Cancer Risks and Clinical Management: If a clinically significant mutation is identified, please see the management tool associated with this report for a summary of cancer risk and professional society medical management guidelines that may be useful in developing a plan for this patient. Testing of other family members may assist in the interpretation of this patient's test result.

Analysis Description: The Technical Specifications summary (https://www.myriadpro.com/documents-and-forms/technical-specifications/) describes the analysis, method, performance, nomenclature, and interpretive criteria of this test. Current testing technologies are unable to definitively determine whether a variant is germline or somatic in origin, which may significantly impact risk estimates and medical management; therefore, these results should be correlated with this patient's personal and family history. The interpretation of this test may also be impacted if the patient has a hematologic malignancy or an allogeneic bone marrow transplant.

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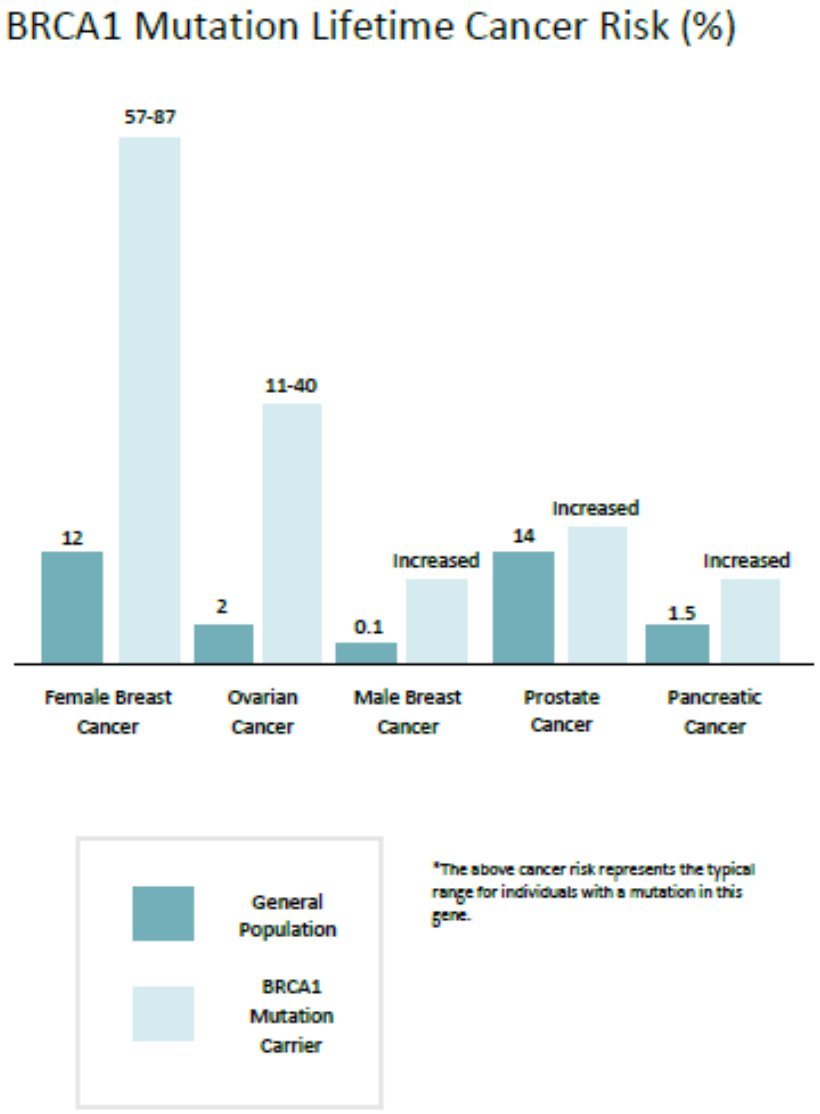
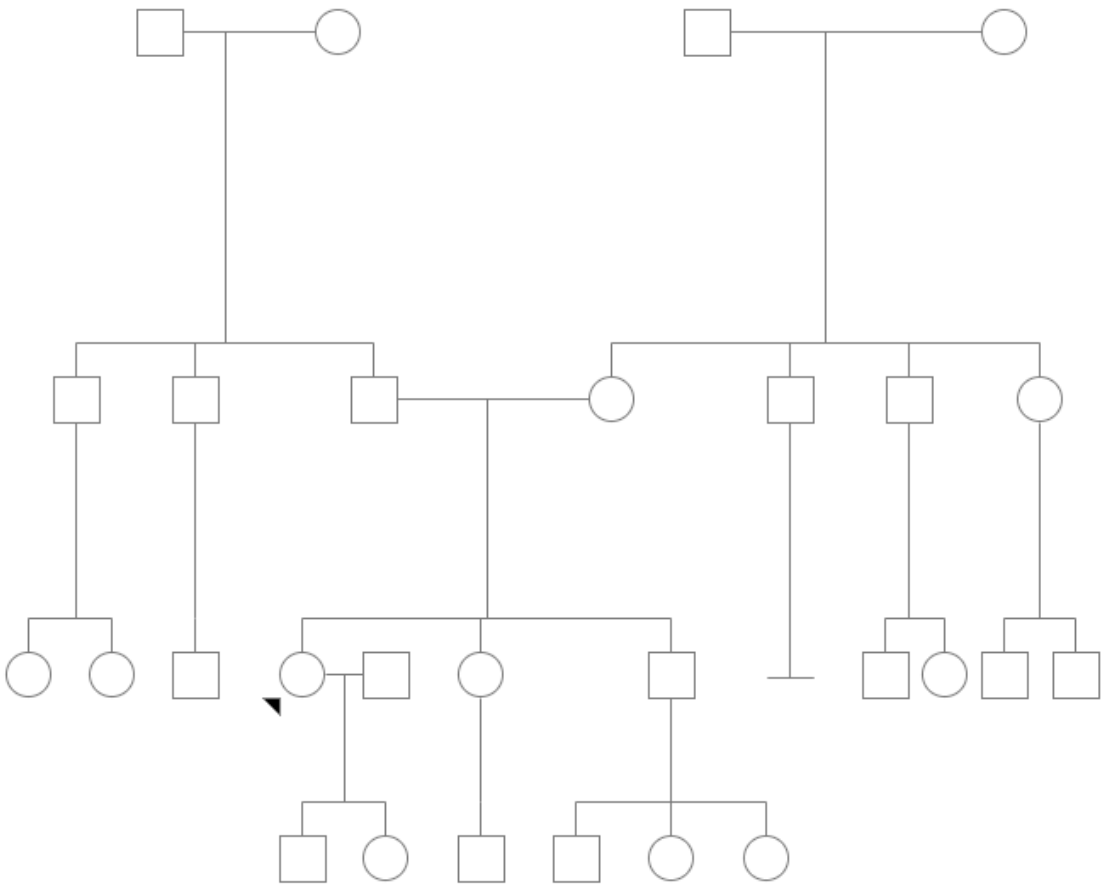
BRCA1 Cancer Risks



- Profile of cancer risks with each tested gene
- Ex. BRCA1 has “primary” cancer risks, as well as “secondary” cancer risks
- Possible that presentation in a specific family is specific to only one type of cancer and/or multiple

TEST FAMILY HISTORY

Paternal Ancestry	Maternal Ancestry



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