Inherited Cancer Predisposition Syndromes

Pinar Bayrak-Toydemir, MD, PhD, FACMG

Professor, Department of Pathology, University of Utah

Medical Director, Molecular Genetics and Genomics, ARUP Laboratories

Outline

- Inherited Breast and Ovarian Cancer syndromes
  - High Risk Genes and syndromes
  - Moderate Risk Genes and syndromes

- Renal Cancer Syndromes and/or Pheochromocytoma
  - Birt-Hogg-Dubé syndrome
  - Von-Hippel-Lindau Syndrome

- Retinoblastoma

- Interesting Cases

- National Comprehensive Cancer Network (NCCN) Guideline for testing and surveillance

Types of genes involved in hereditary cancer syndromes

- Tumor suppressor genes
- Oncogenes

Tumor suppressor genes

- Protein products suppress cell growth
- Recessive at cellular level (loss of normal allele)
- In tumors, germline mutations are accompanied by somatic loss of the normal allele
- Dominant syndromes with incomplete penetrance
- Often also mutated in sporadic cancers

Oncogenes

- Protein products promote cell growth
- Dominant at cellular level (gain of function)
- Rarely inherited (germline)

Breast Cancer Genetics

- Based on twin studies, ~27% of breast cancer results from hereditary factors

- Strong inherited component in 5-10% of breast cancers

- Remaining familial risk likely conferred by combinations of low penetrance alleles

Lalloo and Evans (2012) Clin Genet 82:105-114.

Hereditary breast cancer is defined by

- Onset at a young age
- Bilateral breast cancer
- Multiple primary breast cancers
- A history of first or second- degree family members with similar diagnoses

BRCA1 and BRCA2

BRCA1 and BRCA2 are the most common and well-known causative genes for hereditary breast cancers.

Around 85-90% of hereditary families with both breast and ovarian cancers are caused by mutations in the BRCA1 or BRCA2 genes.

BRCA1 and BRCA2 Mutation Carrier's Cancer Risk by age 70

	General Population	BRCA1	BRCA2
Breast Cancer	12%	45-65%	
Ovarian Cancer	1.5%	39%	~15%
Prostate Cancer	15%	Less than 30%	Less than 39%
Pancreatic Cancer	0.5%	Up to 3%	Up to 7%
Male Breast Cancer	0.1%	Up to 2%	Up to 10%

1. U.S. Preventive Services Task Force. Risk Assessment, Genetic Counseling, and Genetic Testing for BRCA-Related Cancer in Women: U.S. Preventive Services Task Force Recommendation Statement. Ann Intern Med. 2014;160:271-281.
2. Petrucelli, N., Daly, M.B., and Feldman, G.L. (2013). BRCA1 and BRCA2 Hereditary Breast and Ovarian Cancer. *GeneReviews*. Retrieved from: http://www.ncbi.nlm.nih.gov/books/NBK1247/

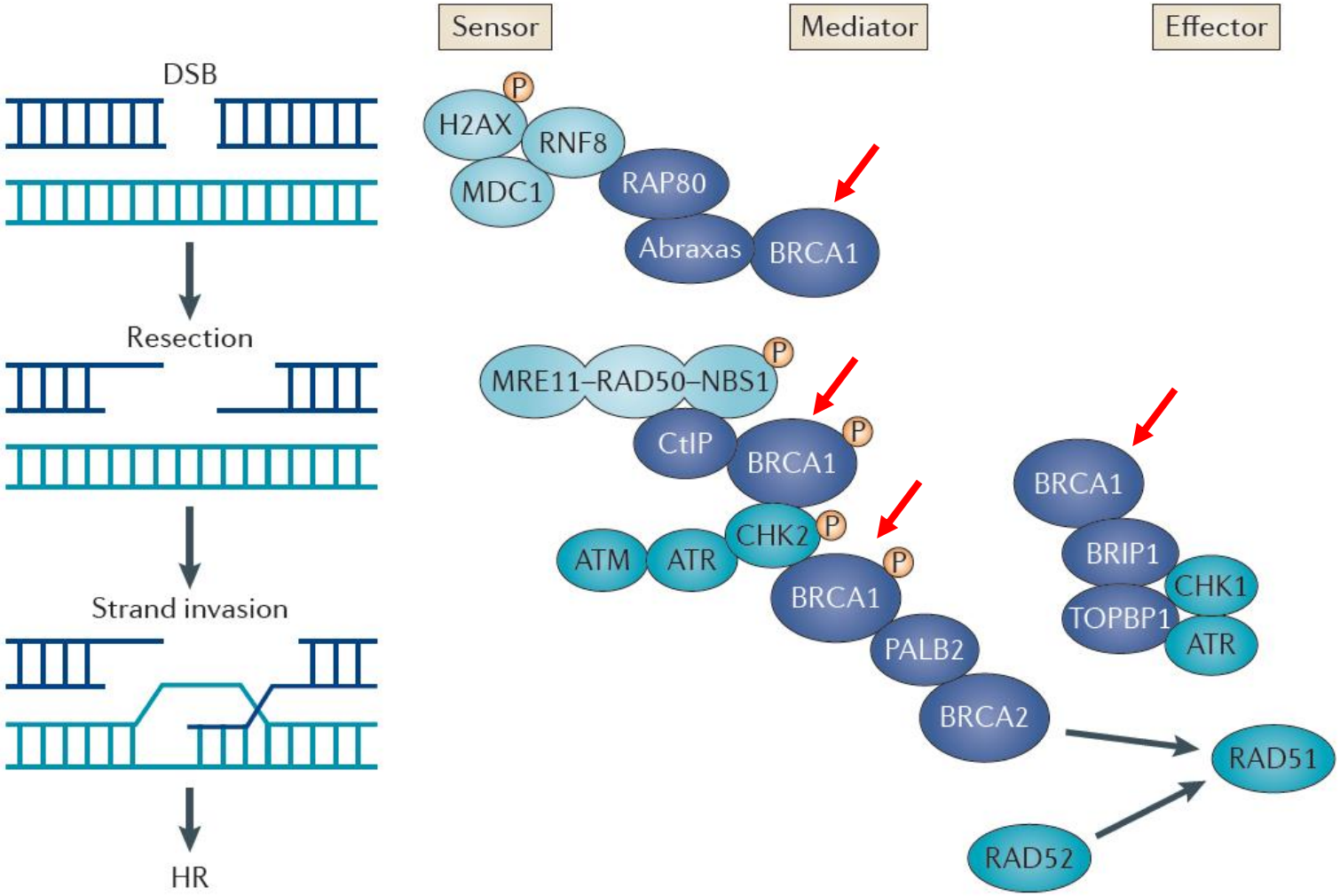
BRCA1 & BRCA2

- Biallelic inactivation in tumors from germline mutation carriers (somatic “second hit”)
- Mutations not common in sporadic breast cancer
- Involved in repair of DNA double strand breaks through homologous recombination

BRCA1 and BRCA2 Proteins

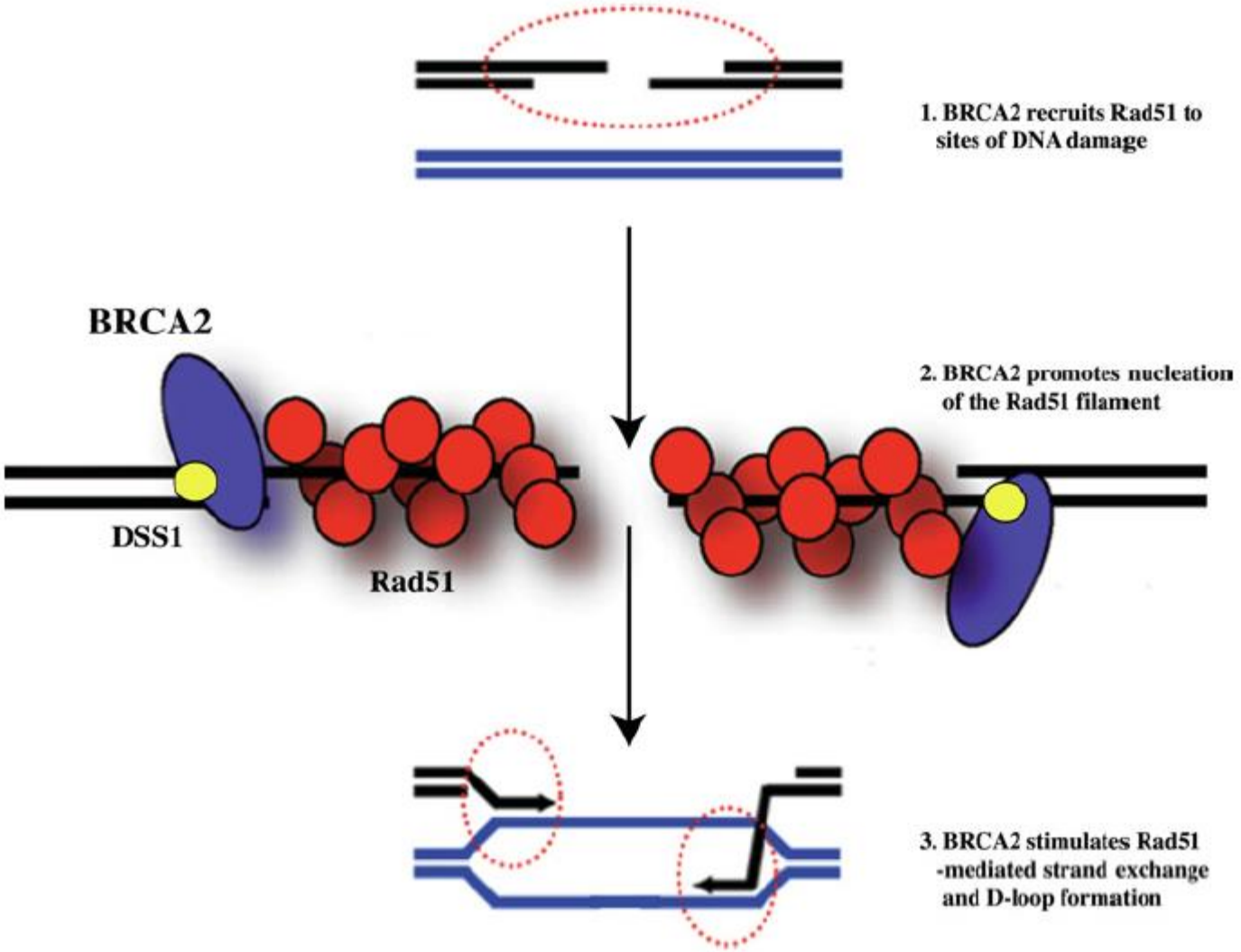
BRCA1:	**Other Proteins**
Recruitment to DNA damage sites	Abraxas
G2/M checkpoint	Abraxas, CtIP and ATM
S phase checkpoint	ATM, BRIP1
Repair during DNA replication	BRIP1
Homolog recombination repair	PALB2
BRCA2:	
Homolog recombination repair	RAD51, DSS1, PALB2

Brca1 function

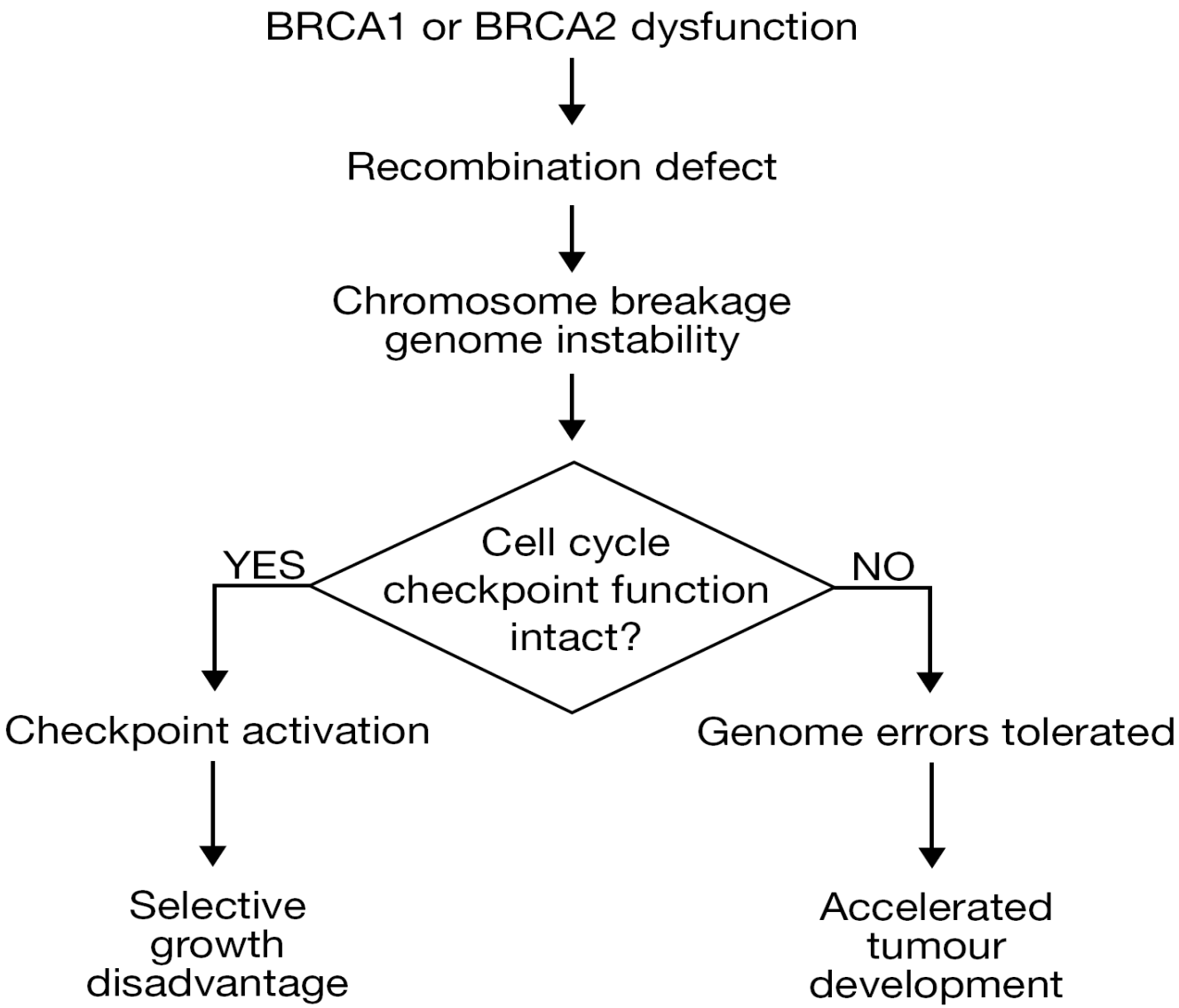


Nat Rev Cancer (2012) 12:68-78

Brca2 function



Biochem Soc Trans (2006) 34:633-45



Nature (2000) 408:429-32

BRCA1 & BRCA2 mutations

- Most mutations novel & truncating (frameshift and nonsense)
- Interpretation of missense mutations difficult
- Large deletions or rearrangements make up 2-5% of disease alleles
- Common mutations in some populations-Ashkenazi-Jewish Population

Variants of Uncertain Significance (VUS) in BRCA1/2 genes

- VUS in ~5-10%

- Predominantly missense mutations in regions of the protein without known function.

- A variety of approaches including conservation, segregation with cancer and population studies are utilized to try and determine the significance.

Databases for Variant Investigation:

- ENIGMA consortium is a ClinVar Expert Panel
- ARUP Lab - Locus Specific Database
- BRCA Exchange-Locus Specific Database

Case 1

~30 years old male

Sister with BRCA2 mutation (not known which mutation)

Maternal aunt with pancreas Ca, maternal uncle with melanoma, maternal grand mother with breast Ca

BRCA1 and BRCA2 Sanger sequencing and deletion duplication analysis

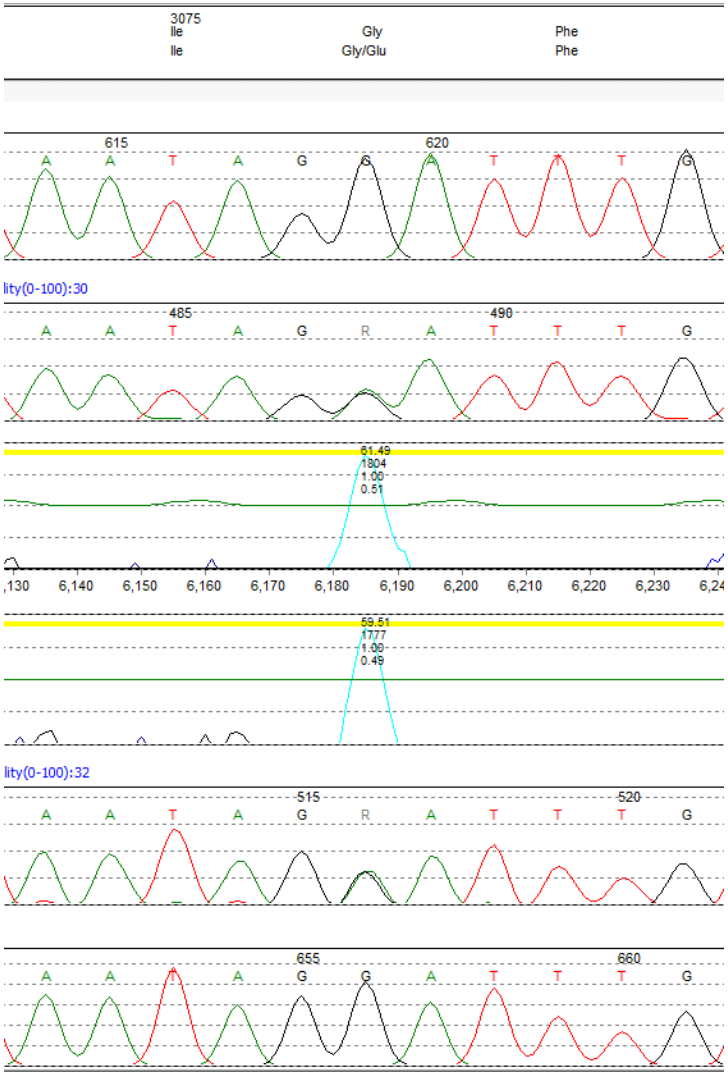
BRCA2 Exon 24

c.9227G>A p.Gly3076Glu

BRCA2 Exon 27

c.10095delinsGAATTATATCT

p.Ser3366frameshift

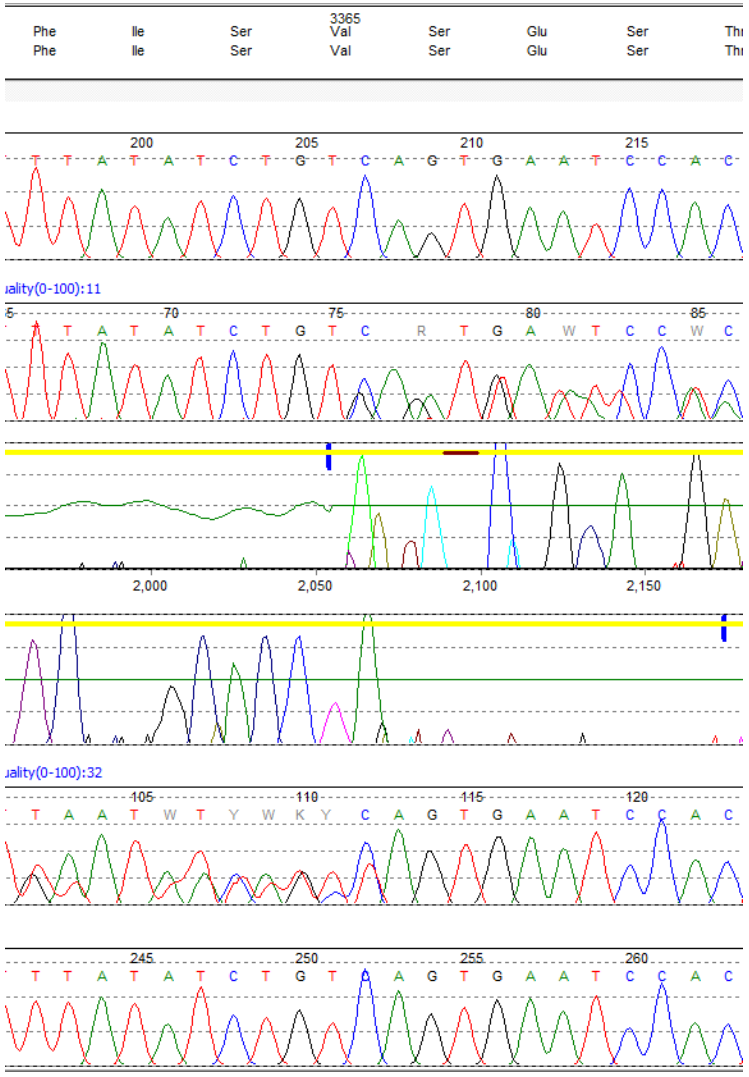


Ref

Forward

Reverse

Ref



Evaluation of c.10095delinsGAATTATATCT; p.Ser3366frameshift BRCA2 variant

- This variant deletes one nucleotide and inserts 11, causing a frameshift, and is predicted to result in a truncated protein.

- Reported in the literature in individuals with breast or ovarian cancer, although it was not demonstrated to be disease-causing.

- In one family, this variant was reported to occur on the same chromosome as a likely pathogenic missense variant.

- Overall allele frequency of 0.04%

- Occurs **in the terminal exon of BRCA2 and at least one upstream truncating variant (p.Lys3326Ter) is considered benign**, suggesting truncating variants downstream of p.Lys3266Ter may be tolerated.

Based on available information, this variant is considered to be **likely benign.**

Evaluation of p.Gly3076Glu variant BRCA2 variant

- Reported in the literature in multiple individuals with a personal and/or family history of breast, ovarian, and/or pancreatic cancer.

- This variant is very rare in population database (one person in 125,000 individuals).

- The glycine at codon 3076 is highly conserved, and functional analyses indicate decreased activity in assays of homology-directed recombination (HDR).

- Other amino acid substitutions at this codon (p.Gly3076Arg, p.Gly3076Val) exhibit decreased HDR activity, and p.Gly3076Val has been reported in a cohort of individual with breast and/or ovarian cancer ; however, the clinical significance of other variants at the same codon has not been conclusively determined.

Based on available information, the p.Gly3076Glu variant is considered to be **likely pathogenic.**

Case 1 - Summary:

Most of truncating variants in the last exon of BRCA2 gene may not be disease causing

Great caution for variant interpretation !

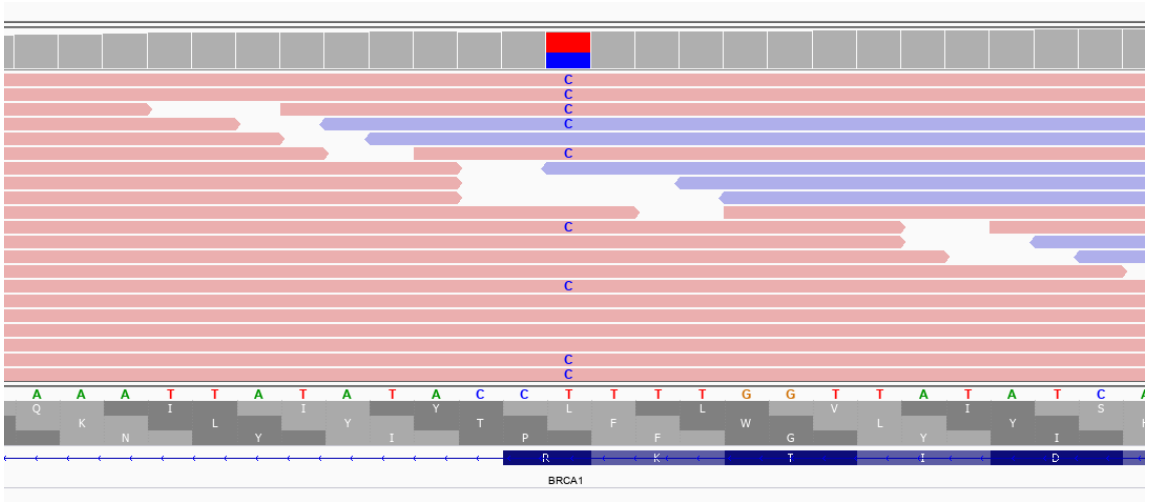
Case 2

~ 20 years old Hispanic female
No cancer
No previous DNA testing
Fam history: mother with breast cancer at age 35, unknown if DNA testing has been performed

23 gene Breast Cancer Gene Panel NGS assay

ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, DICER, EPCAM, MLH1, MSH2, MSH6, NBN, NF1, PALB2, PMS2, PTEN, RAD51C, RAD51D, RECQL, SMARCA4, STK11, TP53

BRCA1
c.211A>G; p.Arg71Gly
Variant Investigation



- Reported in multiple families with a history of breast and/or ovarian cancers.
- This variant is two nucleotide from the canonical splice site.
- Functional characterization indicates aberrant splicing of the BRCA1 transcript, resulting in the introduction of a premature termination codon.
- This variant is reported as pathogenic by multiple laboratories in ClinVar.
- Very rare- seen one in 120,000 individuals

Based on available information, this variant is considered to be pathogenic.

Case 2 - Summary:

Strong component of inheritance- starting at young age

Missense variants may also have spliced defect

NCCN Guideline for Breast and Ovarian Cancer Genetic Testing

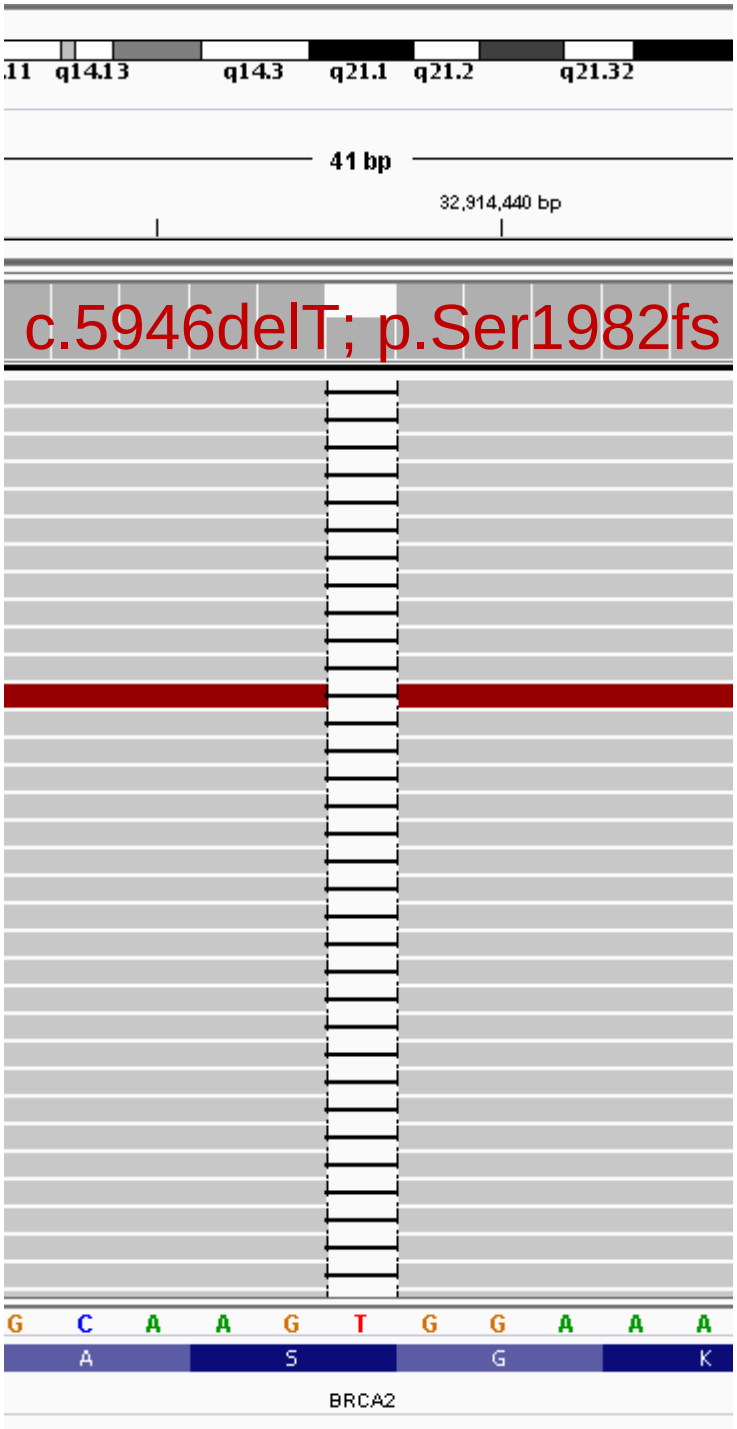
- With known pathogenic mutation in the family
- With known pathogenic mutation on tumor testing
- Breast Cancer diagnosed age =< 50
- Triple negative breast cancer diagnosed age =<60
- Two breast cancer primaries
- Bilateral breast cancer diagnosis =<50 and more than one relative with breast, pancreatic or high-grade metastatic cancer
- Diagnosed at any age with ovarian, pancreatic, met prostate, breast with AJ ancestry, breast with high grade prostate, male breast cancer
- Breast cancer at any age
  - one or more close blood relative with breast cancer age =<50y; or invasive ovarian cancer; or male breast cancer; or pancreatic cancer; or high grade or metastatic prostate cancer
  - two or more close blood relative with breast cancer at any age

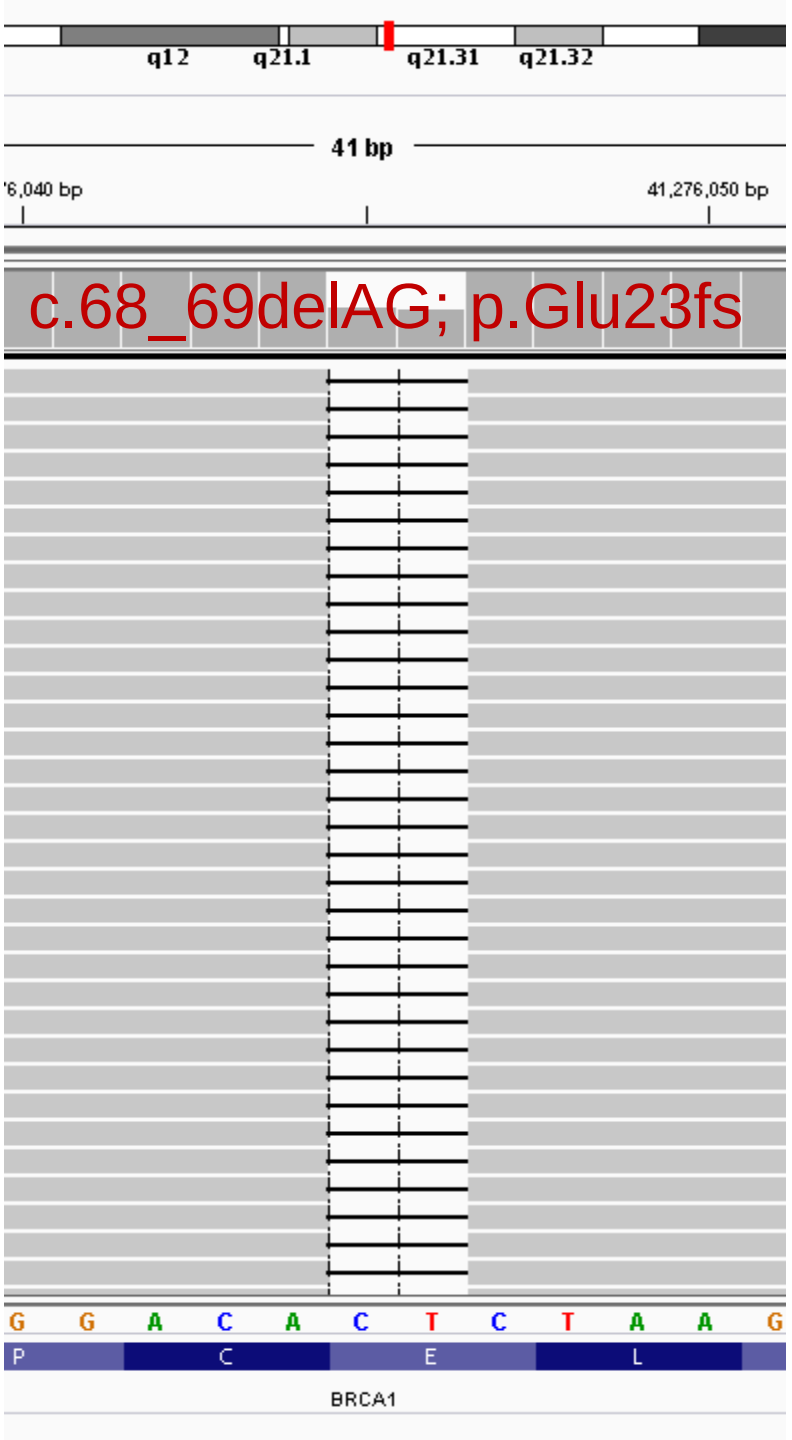
Case 3

~Late 70s female
Caucasian
Diagnosis of breast cancer
No previous DNA testing
Family history unknown
Reason for referral: history of breast cancer

23 gene Breast Cancer Gene Panel NGS assay

ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, DICER, EPCAM, MLH1, MSH2, MSH6, NBN, NF1, PALB2, PMS2, PTEN, RAD51C, RAD51D, RECQL, SMARCA4, STK11, TP53


q14.13
q14.3
q21.1
q21.2
q21.32
41 bp
32,914,440 bp
c.5946delT; p.Ser1982fs
G C A A G T G G A A A
A S G K
BRCA2


q12
q21.1
q21.31
q21.32
41 bp
41,276,050 bp
c.68_69delAG; p.Glu23fs
G G A C A C T C T A A G
P C E L
BRCA1

One pathogenic variant, c.5946delT; p.Ser1982fs, was detected in the BRCA2 gene and one pathogenic variant, c.68_69delAG; p.Glu23fs, was detected in the BRCA1 gene by massively parallel sequencing and confirmed by Sanger sequencing.

Double heterozygosity for pathogenic BRCA1 and BRCA2 variants has been described in the literature in individuals with an earlier onset of breast cancer as well as a more severe disease course.

Other genetic and/or environmental factors may influence severity of clinical phenotype.

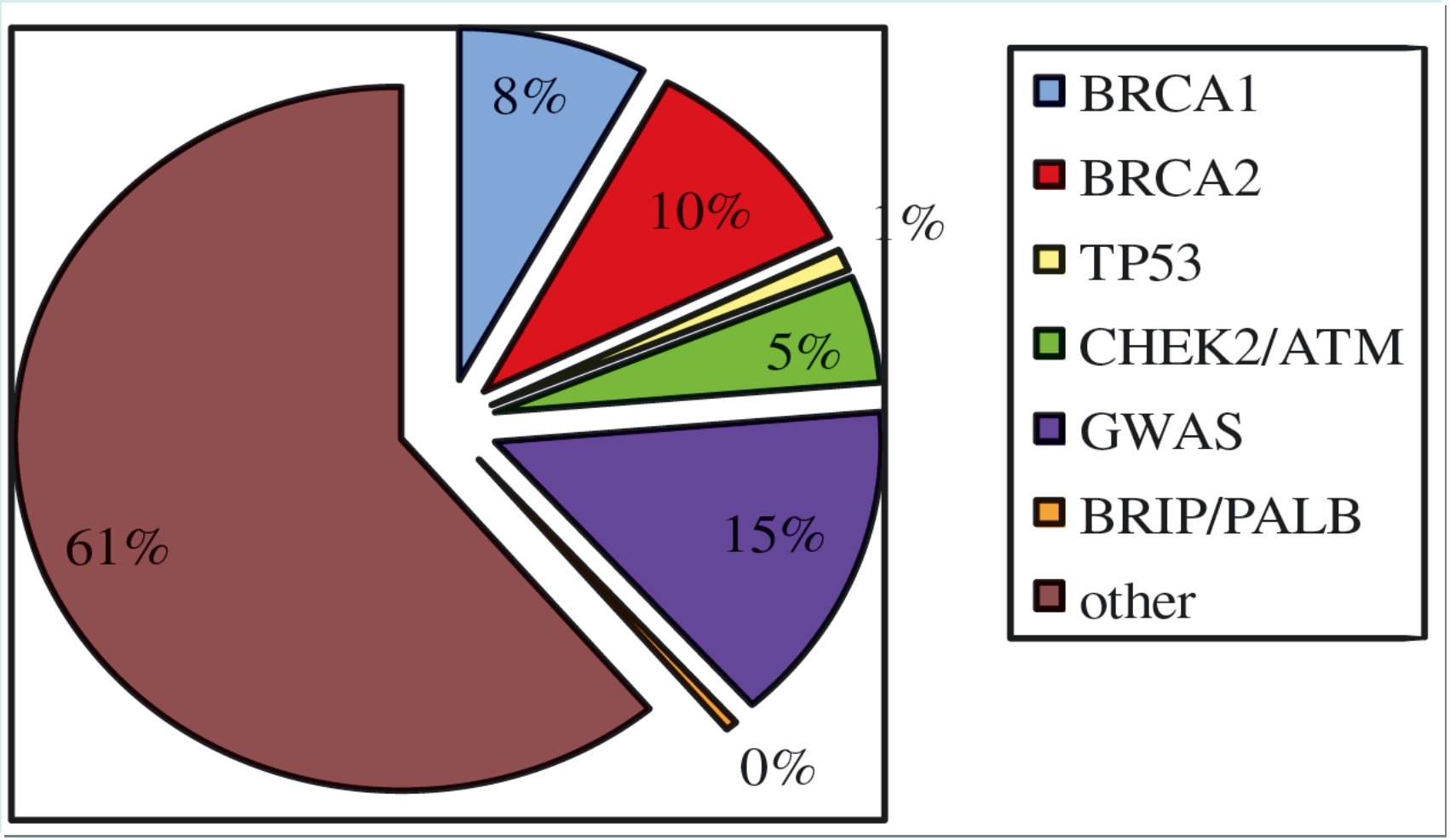
Offspring of this individual have a 50 percent chance of inheriting each of the pathogenic variants.

Case 3- Summary:

Rarely individuals may carry more than one pathogenic variants in high-risk genes

Perhaps some protective genes/alleles for cancer

Hereditary Component of Breast Cancer



Lalloo and Evans (2012) Clin Genet 82:105-114.

High Penetrance genes – BRCA1, BRCA2, CDH1, PALB2, PTEN, TP53

- **Relative Risk (RR=5-10x)**
- **Surveillance and prophylactic surgery**

Moderate penetrance genes – ATM, BARD1, CHEK2, NF1, STK11

- **RR~2-3 fold.**
- **Screening based on family history**

Hereditary Breast Cancer: Moderate penetrance

ATM- Breast and ovarian risk in heterozygous mutation carriers

• Lifetime risk for breast cancer is 6% by age 50 and 33% by age 80 years.

• Relative Risk: ~2.8

• Annual mammogram with breast MRI at age 40

• Certain missense mutations may act dominant negative fashion to increase cancer risk, relative to truncating mutations.

CHEK2

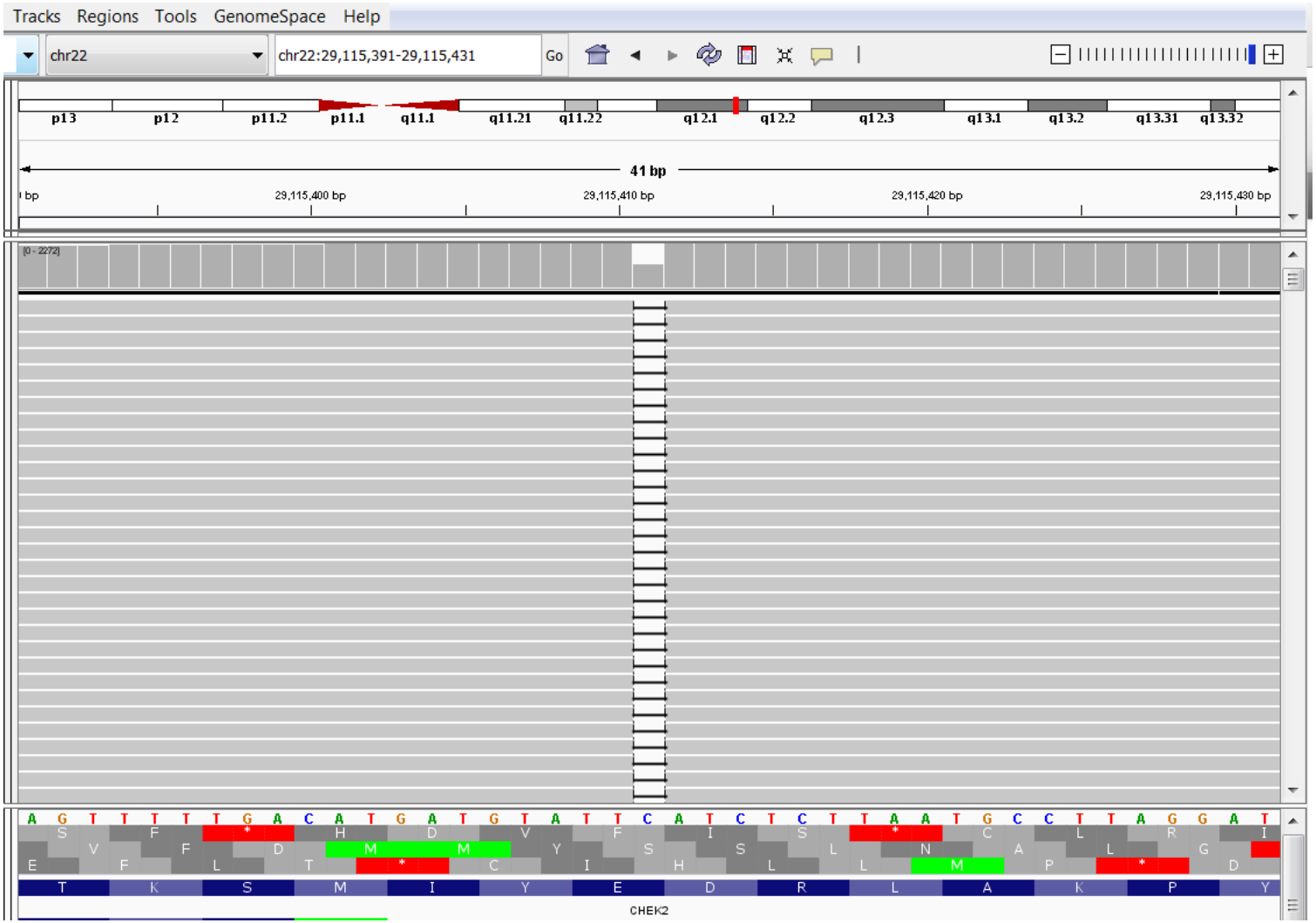
- 1100delC (1% population frequency)
- Relative Risk: ~3.0
- Cumulative lifetime risk for breast cancer is range from 28-37%. Risk is higher in women with stronger family histories of breast cancer than those without.

Case 4

~70 years old female
Caucasian
No cancer
Family history of Breast Cancer (two paternal aunts)

23 gene Breast Cancer Gene Panel NGS assay
ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, DICER, EPCAM, MLH1, MSH2, MSH6, NBN, NF1, PALB2, PMS2, PTEN, RAD51C, RAD51D, RECQL, SMARCA4, STK11, TP53

CHEK2 gene c.655delG mutation



CHEK2 gene c.655delG; p.Glu219fs

- Absent from general population databases

- Has not been reported in the medical literature, however, reported in other laboratories as pathogenic disease -causing variant

- Creates frameshift which introduces an early termination codon in exon 5 and is predicted to result in a truncated or absent protein product.

- The overall contribution of truncating variants in CHEK2 to the development of breast cancer is not well understood, as most identified truncation mutations display some level of incomplete penetrance.

- Heterozygous mutations in CHEK2 have been reported to cause wide range of clinical phenotypes including association with susceptibility to breast cancer. In addition, other genetic and/or environmental factors may influence severity of clinical phenotype.

Case 4 - Summary:

Interpretation of CHEK2 gene variants are very difficult

Interpretation of variants in other moderate risk genes are also very difficult

- High population frequency of variants

- Variable penetrance

High Penetrance Breast Cancer genes –

BRCA1, BRCA2, CDH1, PALB2, PTEN,TP53

High Penetrance genes – BRCA1, BRCA2, CDH1, PALB2, PTEN, TP53

Hereditary Diffuse Gastric Cancer (*HDGC*)

CDH1 encodes E-cadherin

- Involved in cell-cell adhesion
- Roles in signal transduction and cell motility

HDGC Syndrome

~39-52% lifetime risk of lobular breast cancer

~80% lifetime risk for diffuse gastric cancer

Annual mammogram and MRI with contrast starting at age 30

High Penetrance genes – BRCA1, BRCA2, CDH1, **PALB2, PTEN, TP53**

PALB2

- Fanconi Anemia gene
- 0.6-3% of patients with breast cancer carry PALB2 mutations
- RR~5.3
- Breast cancer risk 14% by 50 years, 35% by 70 years of age
- Poorer survival-10 years survival is 48% compared to 72% in BRCA1 mutations

PTEN Hamartoma Tumor Syndrome (*PTEN*)

(Includes Cowden syndrome and other phenotypes)

- Estimated penetrance of PTEN mutations is 80%
- Lifetime risk of breast cancer 25-50% with an average age of 38-50 years old at diagnosis
- Thyroid and endometrial cancer
- Hamartomatous intestinal polyps
- Mucocutaneous lesions (trichilemmomas, acral keratoses)

NCCN Testing Criteria for PTEN Testing

- **Family with known PTEN pathogenic mutation**
- **Personal history of Bannayan-Riley-Ruvalcaba syndrome (**macrocephaly, multiple noncancerous tumors and hamartomas, and dark freckles on the penis in males**)**
- **Meeting clinical diagnostic criteria for Cowden syndrome or PTEN hamartoma tumor syndrome**
- **Adult cerebellar tumors**
- **Autism spectrum disorder and macrocephaly**

Li-Fraumeni syndrome (*TP53*)

- p53: central role in response to DNA damage
- Missense mutations at specific sites are frequent
  (Interfere with protein complex formation)
- Truncating mutations produce milder phenotype
- >90% risk of breast cancer by 70 years
- Also: sarcomas, brain tumors, adrenocortical carcinoma, leukemias

Li Fraumeni syndrome (LFS)

- Autosomal dominant inheritance of cancer susceptibility with proportion de novo mutations.

- Penetrance for women is greater then for men.
  Even when sex-specific cancers are eliminated.
  Breast cancer risk is very high with average age of diagnosis of 32.

- Multiple malignancies in one patient is very common (15%).

Molecular Genetics of LFS

- 80% of LFS families have mutations detectable by sequencing.

- 5-10% have deletions.

- New NCCN guidelines recommend *TP53* testing for any woman with BRCA <age 35 who is *BRCA1/2* negative.

Common Tumor Types in Li Fraumeni Syndrome

- Sarcomas – both soft tissue and osteosarcoma in children and adults, but not Ewing's sarcoma.
- Breast cancer – most common malignancy in LFS families overall with average age of onset ~ 31.
- Leukemias and lymphomas – in both children and adults.
- Adrenocortical carcinoma – in children, otherwise a very rare malignancy. Any child with ACC should have a genetics evaluation.
- Brain tumors – in both children and adults. Choroid plexus carcinoma are highly indicative of p53 mutations.
- GI malignancies including colon cancer.

Syndromes with

Renal Cell Carcinoma and/or

Pheochromocytoma

Hereditary papillary RCC (*MET*)

- Oncogenic mutations

Birt-Hogg-Dube Syndrome (FLCN)

- Chromophobe Renal Cell Carcinoma
- Cutaneous hamartomas (fibrofolliculomas)
- Lung cysts / spontaneous pneumothorax

Case 5

~ 60 year old female with mechanical mitral valve and congestive heart failure

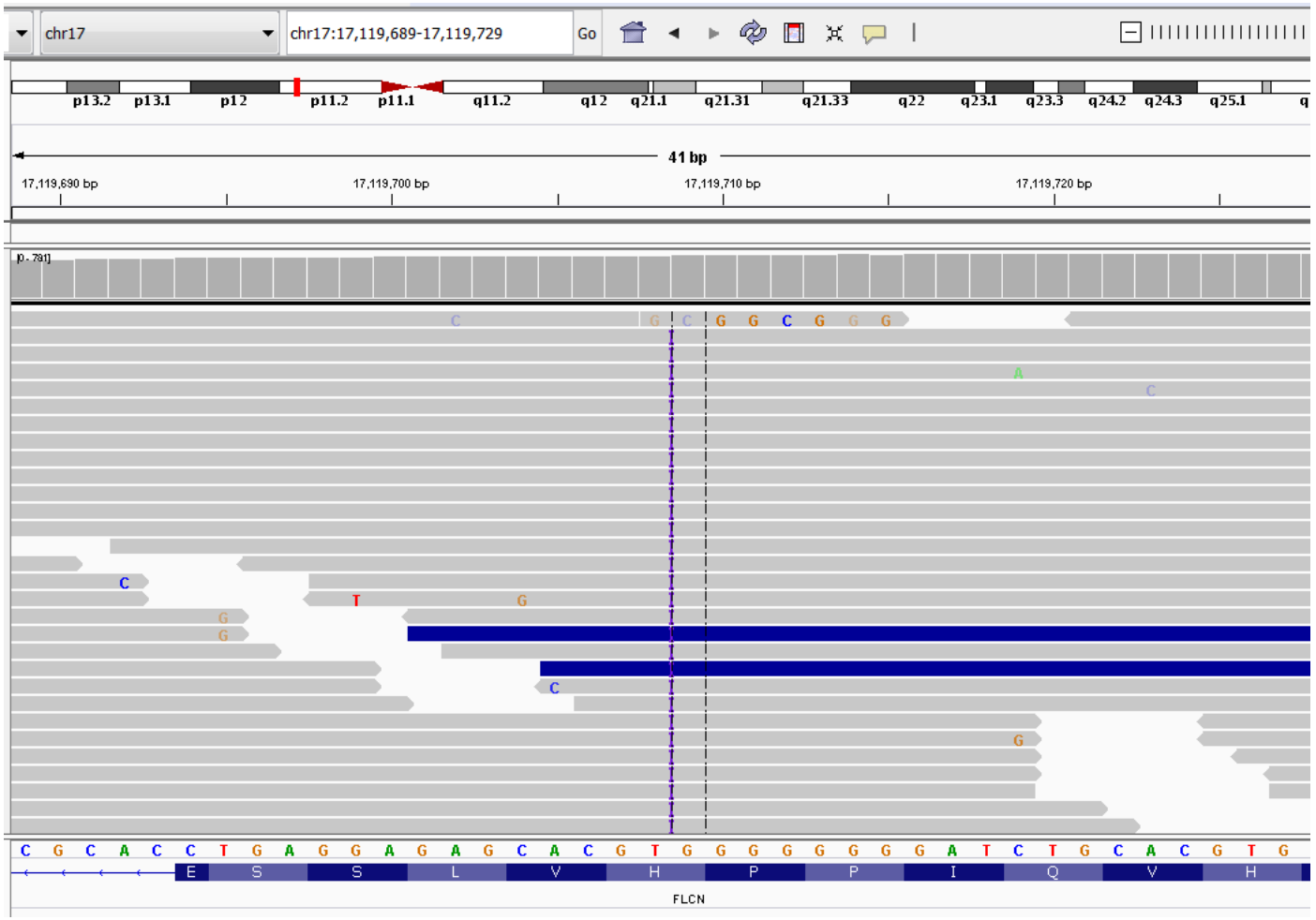
- Left ventricular systolic function is severely reduced, cystic lungs bilaterally, moderate cardiomegaly, dense lesions in liver possible a lipoma or angiomyolipoma

- Multiple lung cysts

21 gene Renal Cancer Panel:
BAP1, DICER1, EPCAM, FH, FLCN, MET, MLH1, MSH2, MSH6, PMS2, PTEN (promoter included), SDHA, SDHB, SDHC, SDHD, SMARCA4, SMARCB1, TP53, TSC1, TSC2, and VHL

Next generation sequencing and exonic level deletions/duplications analysis

Pathogenic Variant:
Gene: FLCN
Nucleic Acid Change: c.1285dupC; Heterozygous
Amino Acid Alteration: p.His429fs



Interpretation:

One pathogenic variant, c.1285dupC; p.His429fs, was detected in the FLCN gene by massively parallel sequencing and confirmed by Sanger sequencing.

The c.1285dupC, is a well-known pathogenic variant that occurs in the mutational sequence hot-spot of eight cytosine nucleotides in exon 11 on the FLCN gene.

Pathogenic FLCN variants are associated with Birt-Hogg-Dube syndrome, an autosomal dominant hereditary cancer syndrome. The syndrome can present with a high degree of clinical variability. Offspring of this individual have a 50 percent chance of inheriting the variant.

Recommendations:
Genetic consultation is indicated, including a discussion of medical screening and management. At-risk family members should be offered testing for the identified pathogenic FLCN variant.

Case 5 - Summary:

- Clinical diagnosis of Birt-Hogg-Dube syndrome is very difficult
- Patients can go to emergency service with only lung symptoms

Von Hippel-Lindau Syndrome (*VHL* gene)

- Clear cell Renal Cell Carcinoma
- CNS & retinal hemangioblastoma
- Pheochromocytoma

- Truncating mutations: low risk of pheochromocytoma

- Missense mutations: high risk of pheochromocytoma and variable risk of Renal Cell Carcinoma

Retinoblastoma

- 1 in 20,000 children affected
- Unilateral or bilateral tumors develop in early childhood
- Occurs in heritable and non-heritable forms

Only 20% of patients with bilateral Rb have a family history

80% are de novo mutations without family history

Overall 90% penetrance for diagnosis of Rb in mutation carriers.
Low penetrant mutations including missense and splice site are seen.

RB1 gene encodes a cell cycle regulator. Inhibits the G1 to S phase by recruiting histone deacetylases to promoters to inhibit transcription of genes required for S phase. RB1 protein is regulated by phosphorylation.

Constitutional RB1 mutations –variety of null mutations

- Cytogenetically visible deletions (<5%)
- Small deletions (detected by MLPA)
- Nonsense and truncating mutations (80%)
- Missense mutations

Testing approach:

- Bilateral - analyze RB1 directly from blood.
- Unilateral - analyze RB1 in tumor AND blood.
- Identify both “hits” in the tumor and then see if one is found in the blood.

Results of Two Unilateral patients

Hereditary form of Rb

Sample	Allele 1	Allele 2
Tumor 1	Q347X	LOH
Blood 1	Q347X	Normal

Sporadic Rb due to somatic mutation/methylation

Tumor 2	Methylation Promoter	567delAG
Blood 2	Normal	Normal

Genetic Counseling

- All bilateral cases considered constitutional with 80% *de novo* cases. Parents should have dilated eye exam to look for retinocytomas as well as genetic testing.

- 13-15% of unilateral cases are constitutional.
No clear predictor of which unilateral cases have constitutional mutations.

- Germline mosaicism in parents of bilateral cases leads to 7% recurrence risk for unaffected parents of bilateral cases.

- Allele-specific PCR analysis for 11 recurrent mutations identified that 4.5% of retinoblastoma probands are mosaic for the mutation (similar rates for unilateral and bilateral). Rushlow, *Human Mutation*, 2009.

Recurrence Risk for Rb in absence of testing

Clinical scenario	Retinoblastoma Risk
Offspring of bilateral cases	45%
Offspring of unilateral cases	7.5%
Sibling of bilateral cases (with unaffected parents)	5-7%
Sibling of unilateral cases (with unaffected parents)	1%

Thank you very much !