Understanding the Functional Significance of Variants Identified in Breast Cancer Susceptibility Genes

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Shyam K. Sharan, Ph.D.
Mouse Cancer Genetics Program
Center for Cancer Research, NCI

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BRCA1 and BRCA2 Mutation Increases Breast Cancer Risk

Responsible for most familial breast cancer

Lifetime risk in general population: 13.7%
BRCA1/2 mutation carriers: 35-85%

Increased ovarian cancer risk

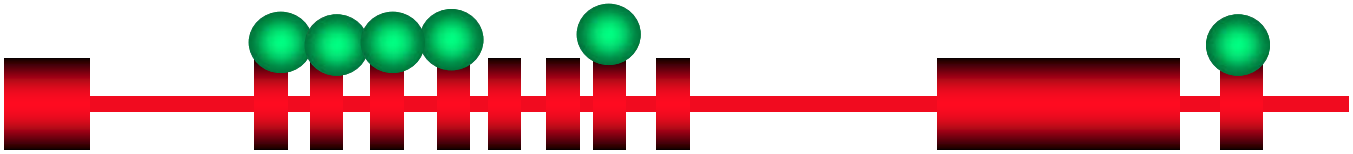
BRCA1 and BRCA2: Role in DNA Repair

BRCA1: 1863 amino acids

RING Finger
E3 ligase

BRCT-repeat
Transcriptional activation

BRCA2: 3418 amino acids



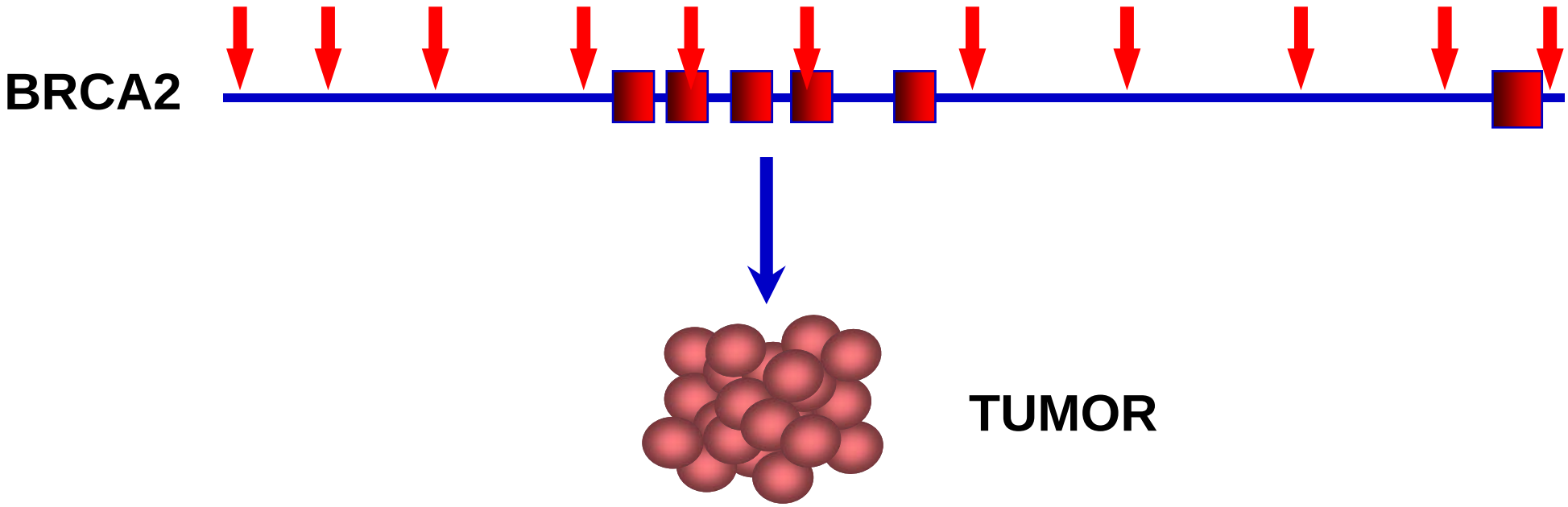
BRC Repeats

Several ss-DNA
binding domains

RAD51

Missense Mutations in *BRCA1* and *BRCA2*

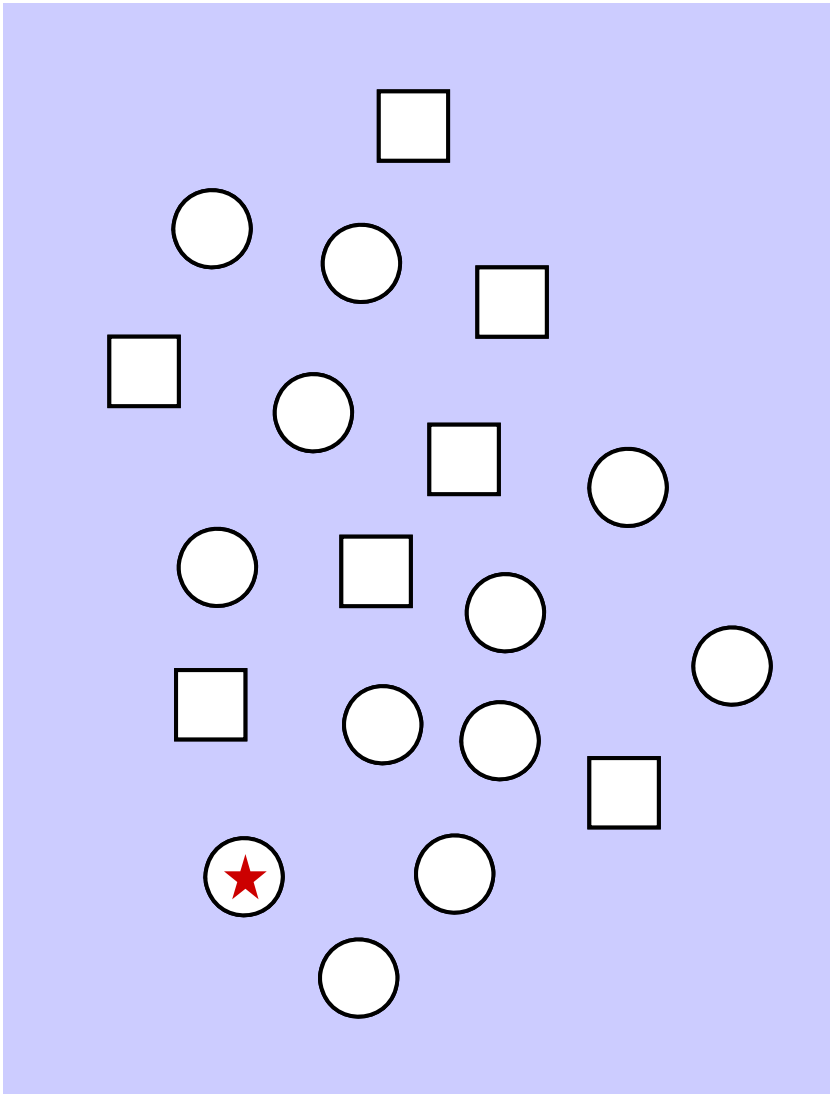
Single amino acid variants:
24% in *BRCA1* ; 47% in *BRCA2*

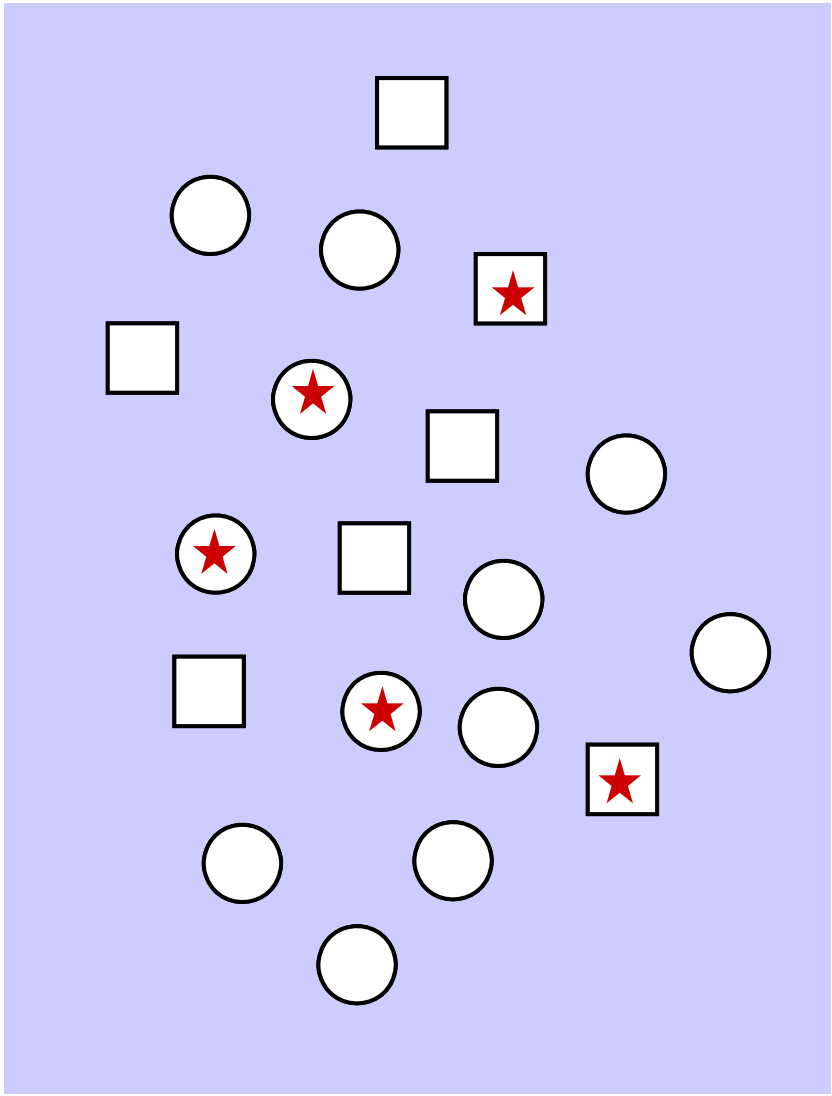


Functional Significance of Single Amino Acid Changes in *BRCA1* and *BRCA2*: Neutral or Deleterious?

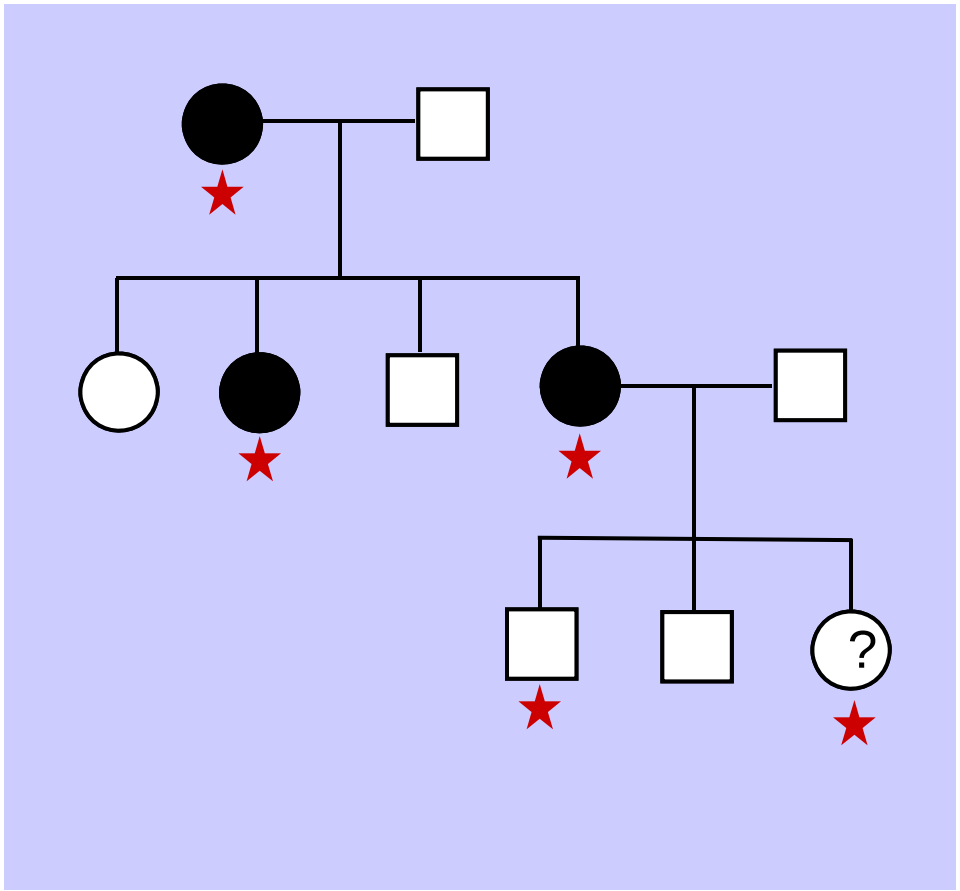
- *Several predicted missense mutations were found to be neutral changes*
- *No reliable functional assay*
- *Prevalence in the general population*
- *Co-segregation with disease*

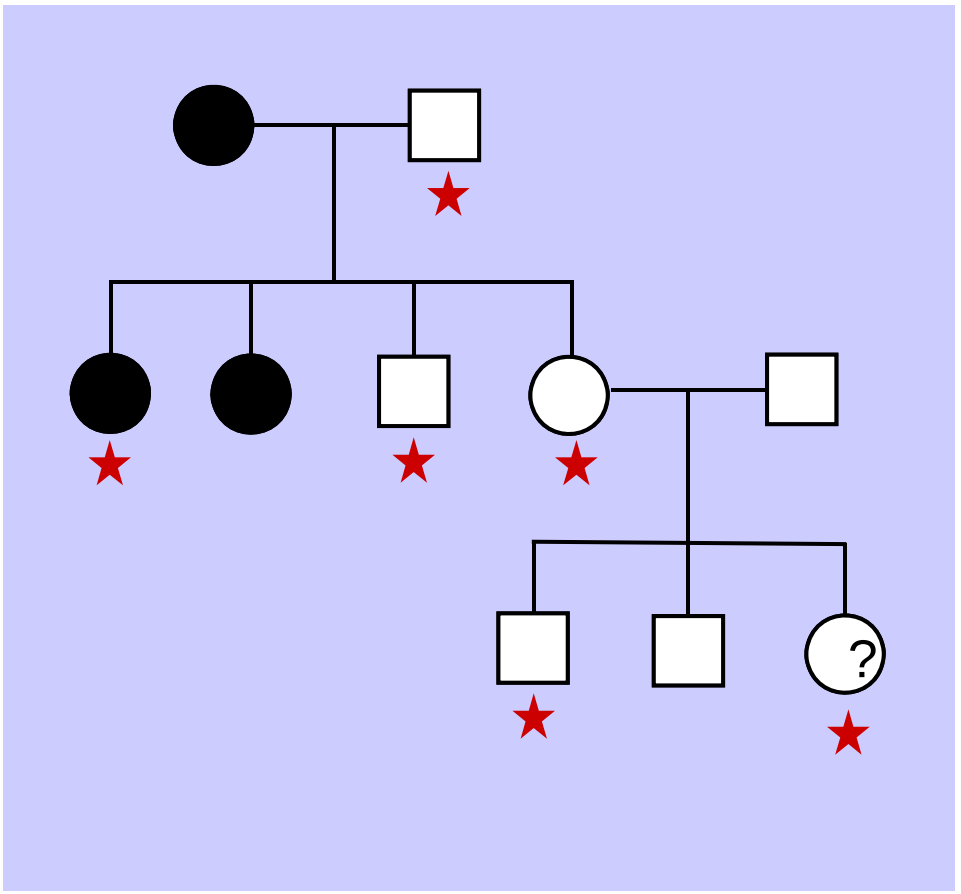
Prevalence in the General Population





Linkage Analysis





Prevalence of *BRCA1 & BRCA2* Mutations

- *10,000 individuals were screened for BRCA1 and BRCA2 mutations by Myriad Genetics*.*
- *55% indicated a personal history of breast or ovarian cancer*
- *17% had deleterious mutations*
- *13% had one or more variants of unknown clinical significance*
- *How about other genes?*

Journal of Clinical Oncology (2002)* ***20,* *1480-1490.*

A Mouse Embryonic Stem Cell-Based Functional Assay

- Embryonic Stem cells for functional analysis

  BRCA1/2 are essential for ES cell viability, maintain stable genome

- Use of Bacterial Artificial Chromosomes (BAC) containing human *BRCA1 or 2*

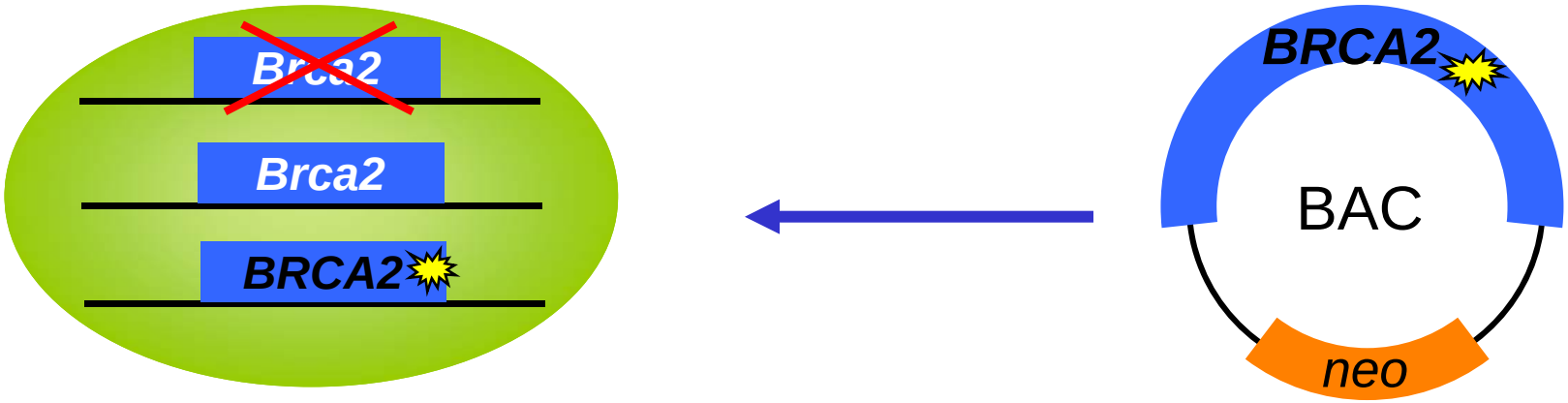
  Average insert size of BAC is ~150,000 bases coding & non-coding alterations, expression at physiological levels, easy to modify by recombineering

Recombineering: Recombination-based Genetic Engineering

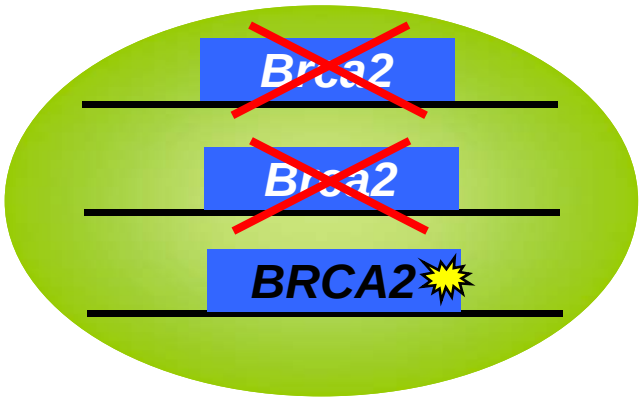
- Technology developed at NCI
- Utilizes the recombination system of bacteriophage
- Extremely efficient method to manipulate DNA
- Allows precise and rapid alteration of a single nucleotide

Swaminathan et al., Genesis, 2001;
Yang and Sharan, Nucleic Acids Res. 2003;
Sharan et al., Nature Protocols, 2009

Functional Analysis of BRCA2 in Mouse ES Cells



Functional Analysis of BRCA2 in Mouse ES Cells



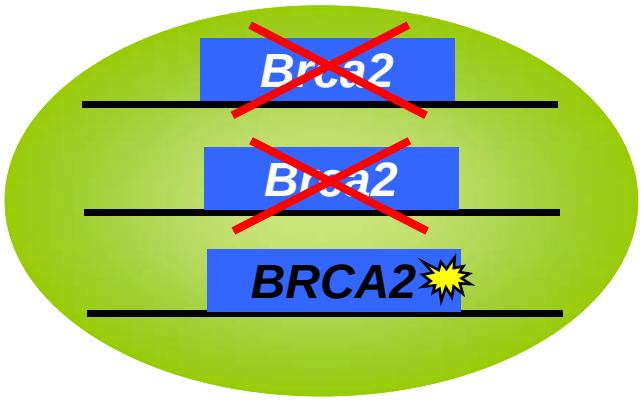
ES cells are not viable

Mutation is deleterious

ES cells are viable

Mutation can be neutral or hypomorphic

Examining BRCA2 Function in Viable ES Cells

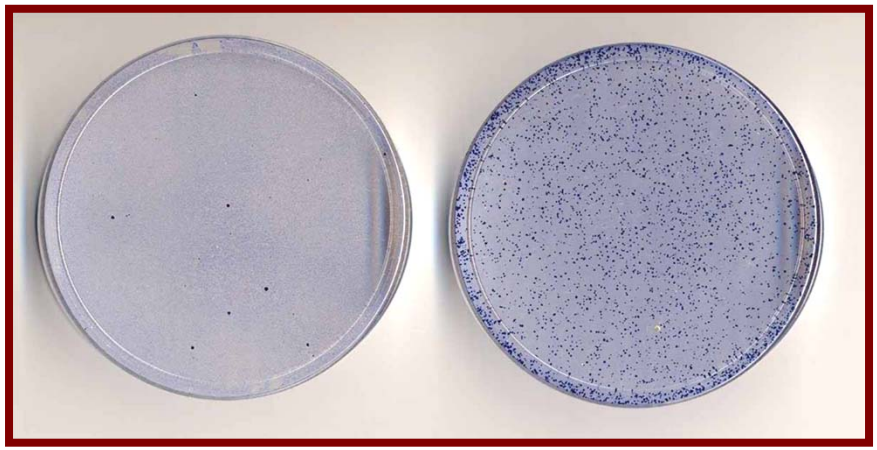


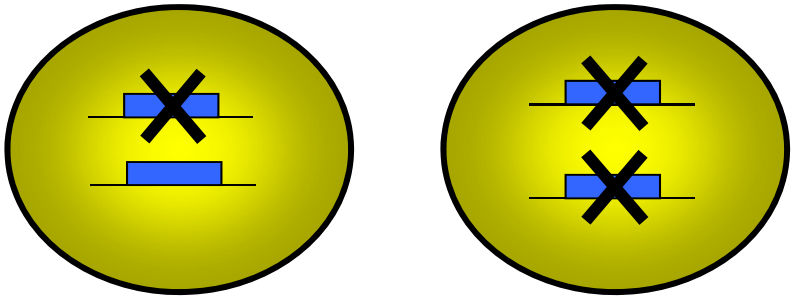
- Test ability to repair damaged DNA
- Effect on overall genomic stability

Functional Evaluation of BRCA2 Variants in Mouse ES cells

BAC Complementation

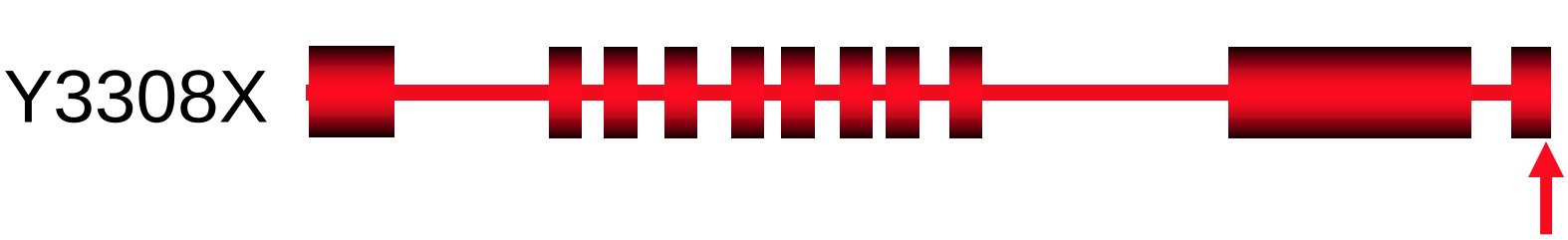
Brca2 Ko/+ ***Brca2 Ko/Ko***

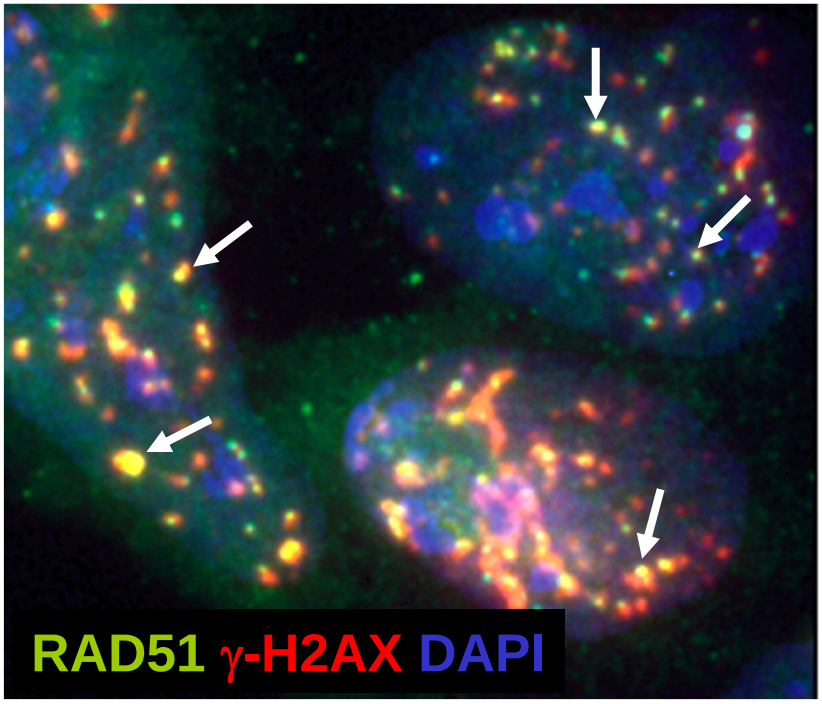




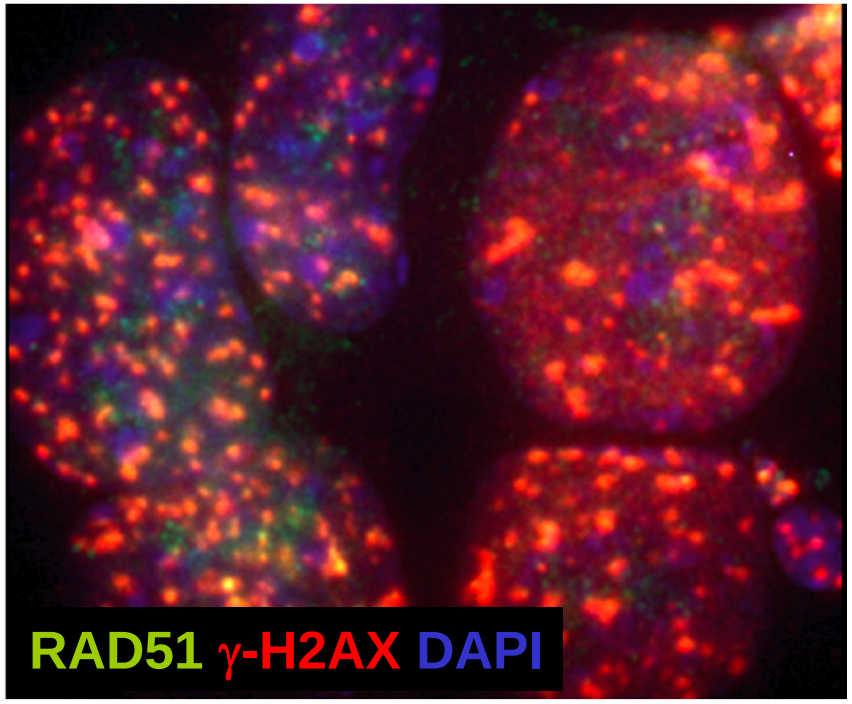
Sergey Kuznetsov

Y3308X Mutant cells are Viable but Hypersensitive to Genotoxins





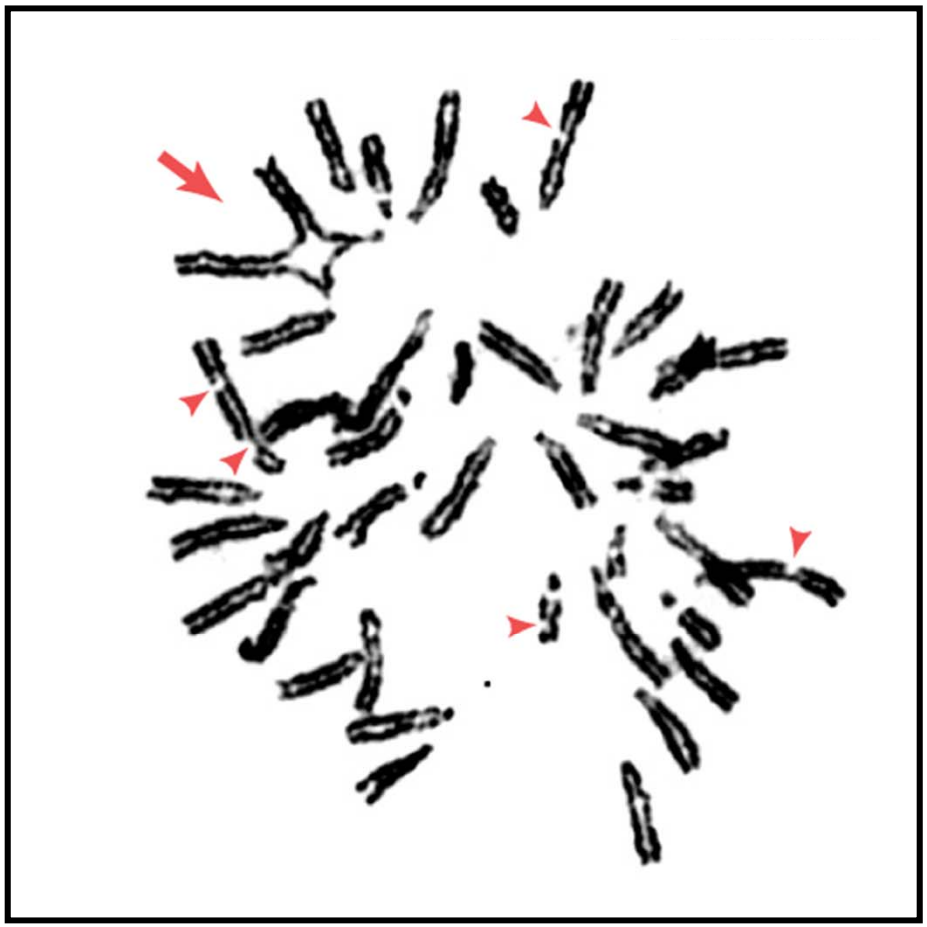
Brca2 Ko/+



Y3308X

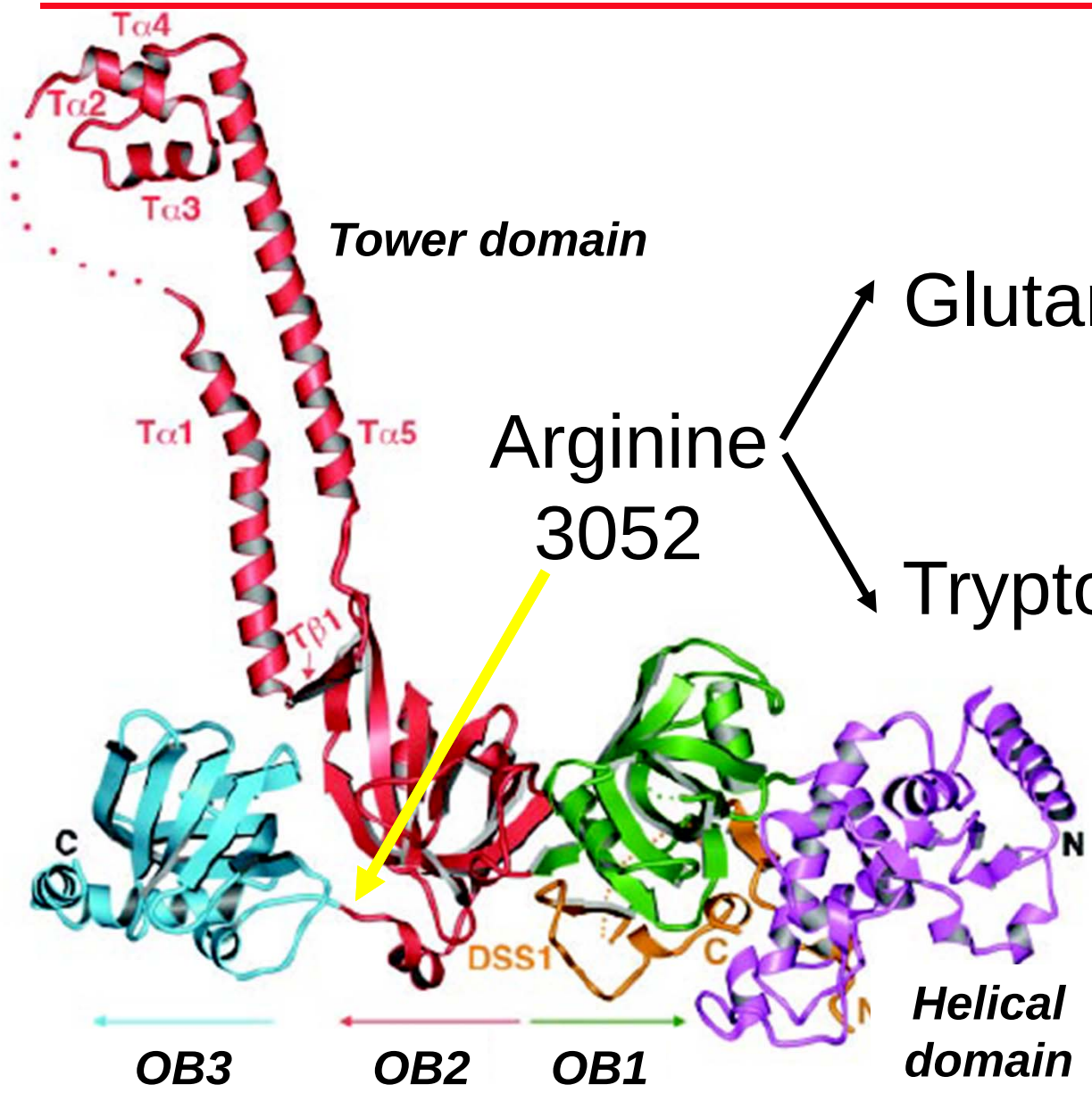
Y3308X cells Exhibit Genomic Instability

Brca2 Ko/+



Y3308X

BRCA2: Structure-Function Analysis



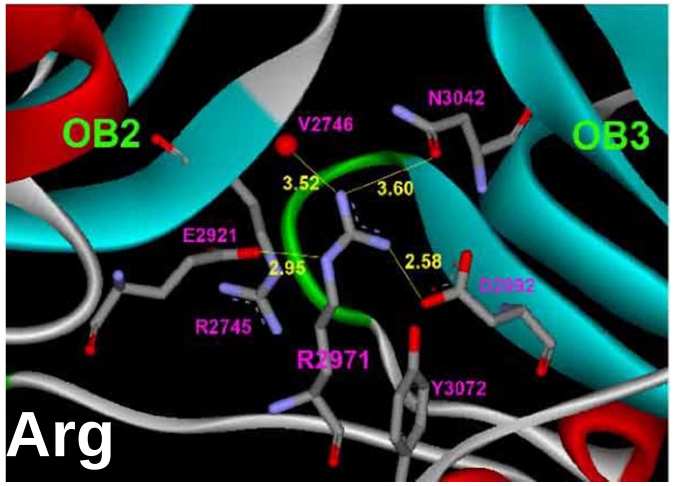
Arginine 3052

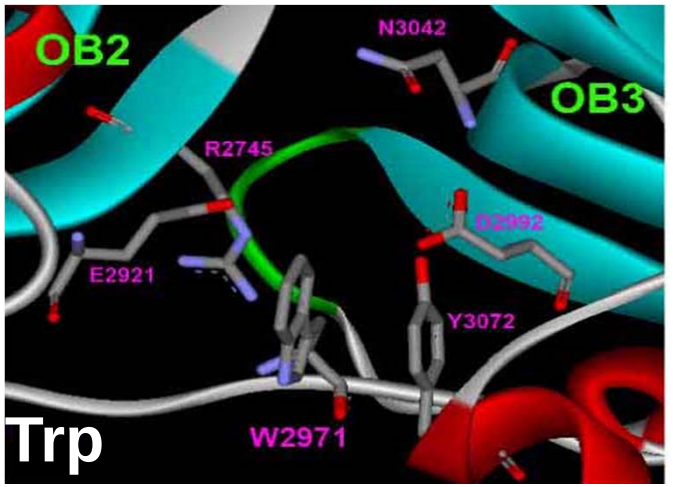
Glutamine

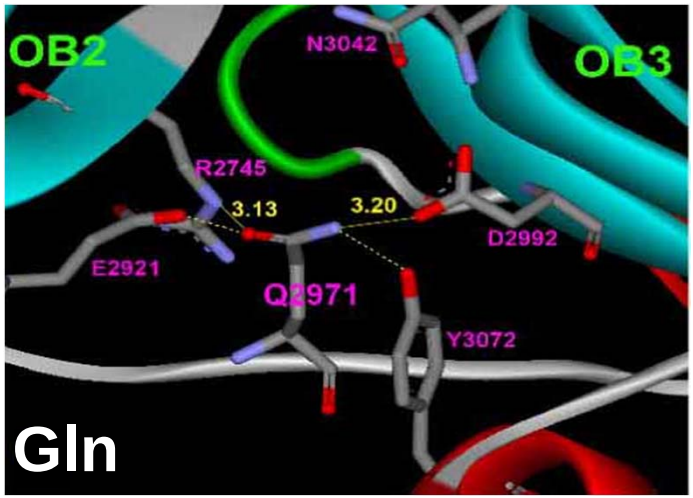
Tryptophan

C-Terminal Domain of BRCA2

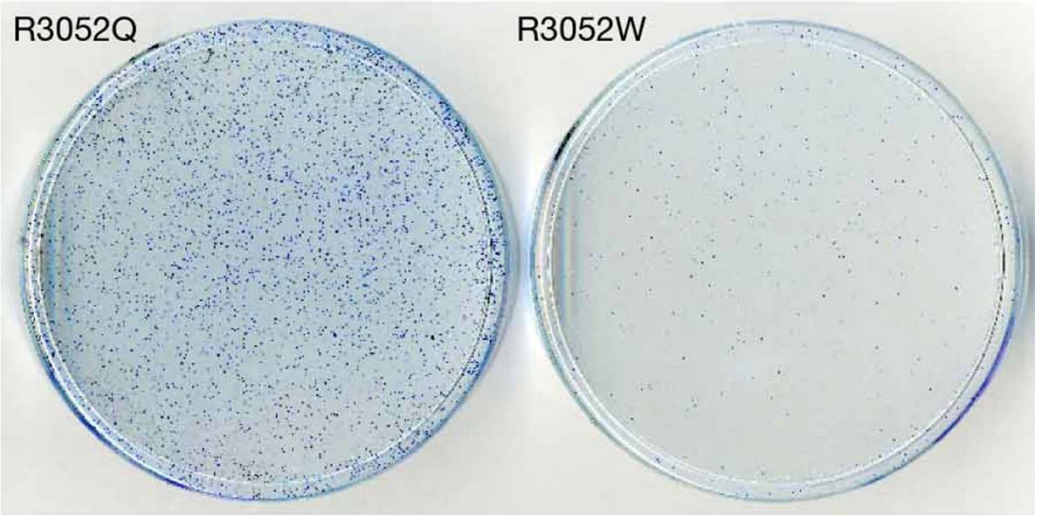
BRCA2 Structure Analysis
Arg3052Trp vs. Arg3052Gln





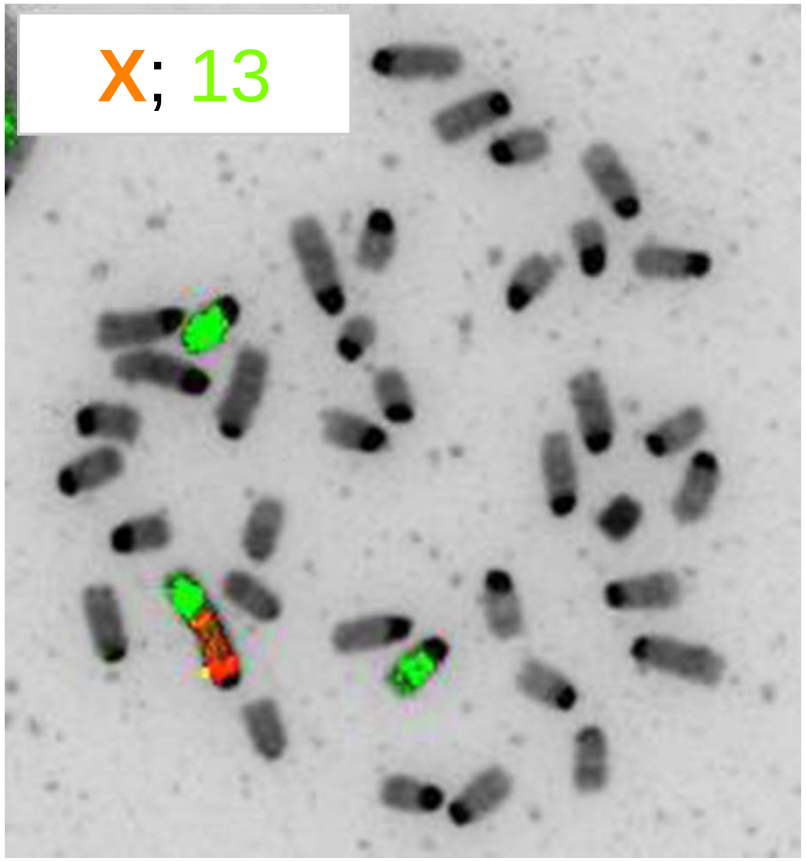


R3052Q Variant Shows Moderate Sensitivity to DNA Damaging Agents



Arg3052Gln Arg3052Trp

R3052Q Variant Results in Moderate Genomic Instability



Evaluating Functional Significance of Missense Mutations in ES cells

Unclassified variants
1100 BRCA2

Clinical relevance
Evolutionarily conserved
Functional importance

20 Variants

13 Neutral

4 ES cell lethal

3 Hypomorphic

Kuznetsov et al. *Nature Medicine* 2008

Functional Analysis of *BRCA1* mutants in ES cells

RING domain BRCT domain

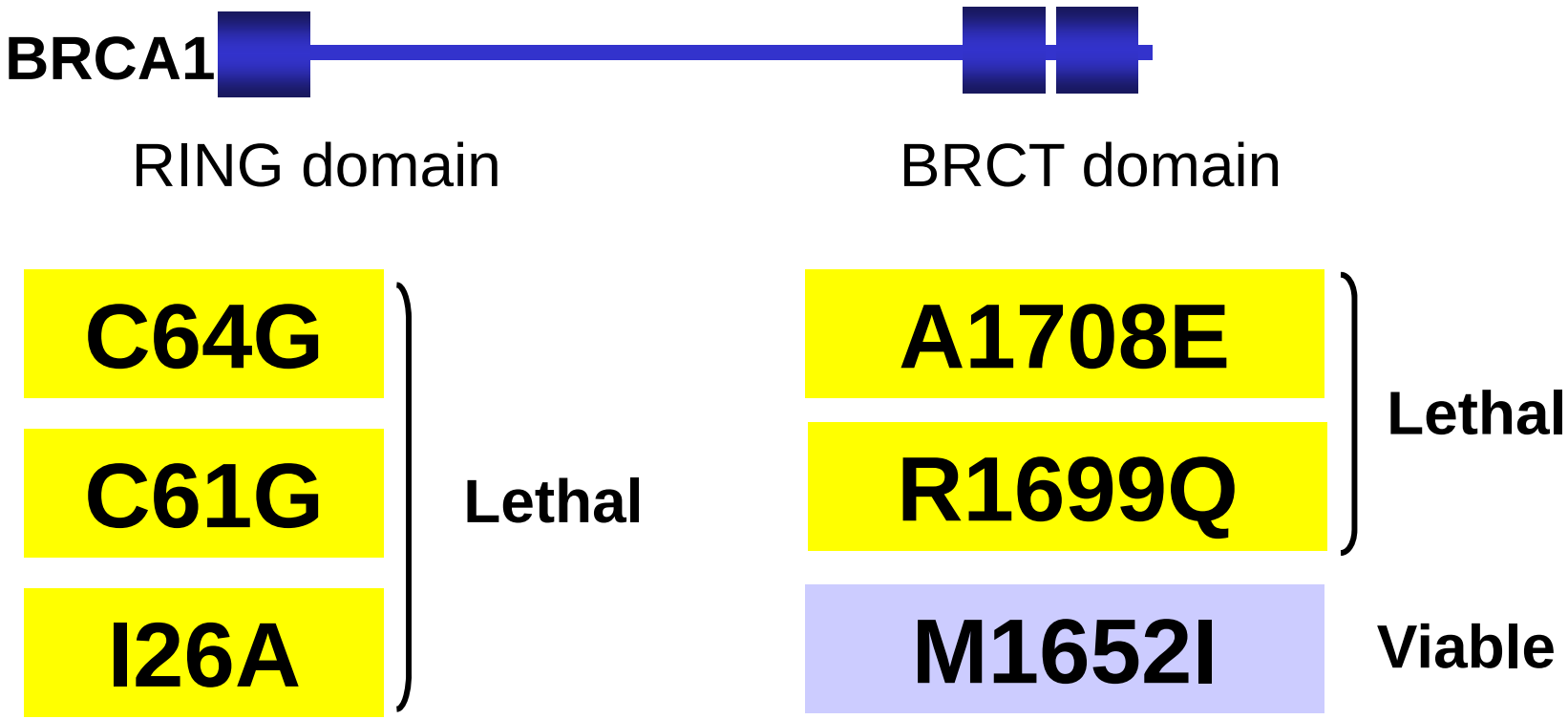
Clinical relevance
Evolutionarily conserved
Functional importance

15 Mutations

RING Domain | Phosphor. sites | BRCT Domain

Chang et al. *J Clinical Investigation* 2009

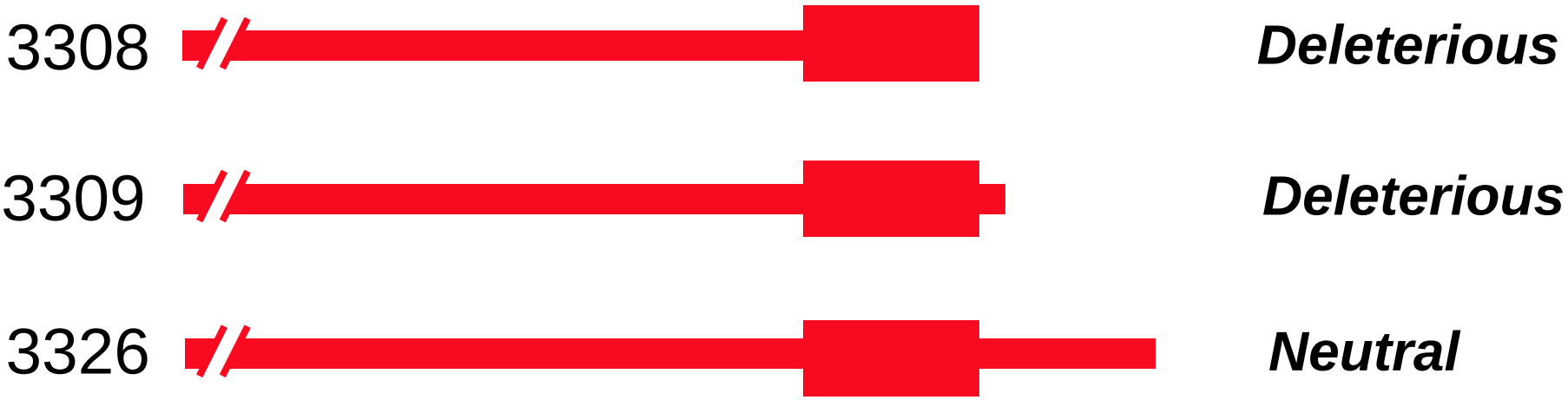
Functional Analysis of Deleterious Variants in Mice



Suhwan Chang

E3309X: a Real Life Dilemma

Truncations at codon:



Conclusions

- *ES cells provide a simple, tractable system to study BRCA1 & BRCA2 variants*
- *Clinical relevance*
- *The ES cell-based approach can be used to examine variants identified in other human disease genes*

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