

# Standards and Guidelines for the Interpretation of Sequence Variants

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2019 11 8

# Question

- Can our lab classify novel missense variant into pathogenic or benign, not VUS?

# **Standards and Guidelines for the Interpretation of Sequence Variants**

- To describe variants identified in Mendelian disorders
- American College of Medical Genetics and Genomics (ACMG) [1]
- ENIGMA BRCA1/2 Gene Variant Classification Criteria
- International Agency for Research on Cancer (IARC)

# Why is BRCA1/2 special?

- High prevalence in population
- Frequent benign variant

# **What about hereditary breast and ovarian cancer syndrome (HBOCS)**

- BRCA1/2 and other genes
- Breast, ovarian cancer and other cancers
- Prevalence (between 1 in 200 to 1 in 800 people)
- Penetration rate (40-90%)

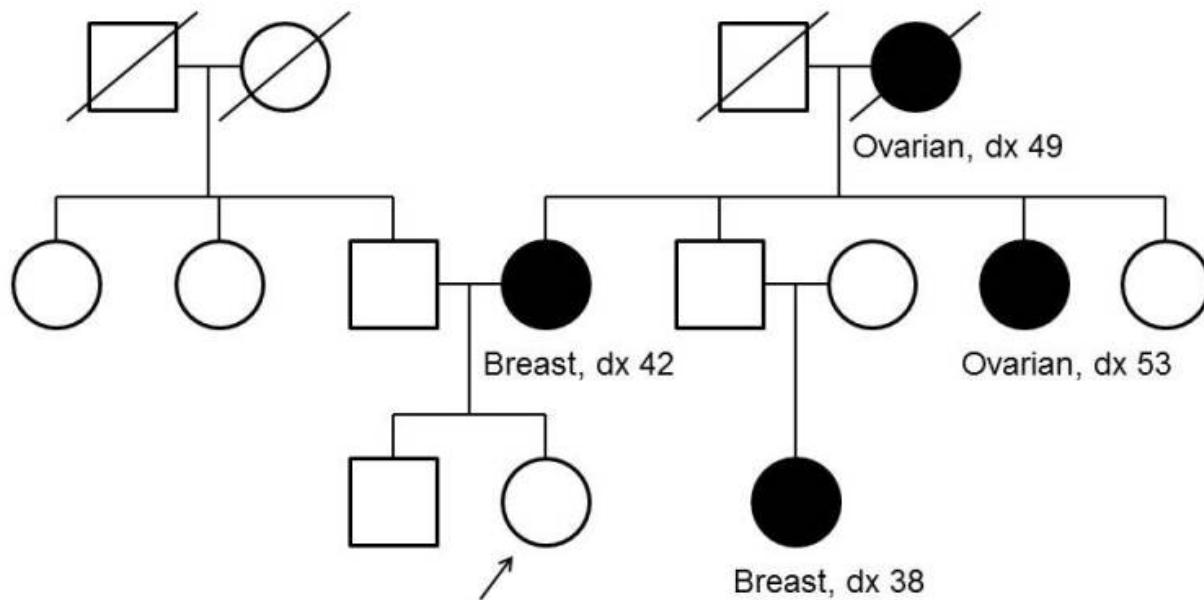
# Categories of interpretation of variants

- Pathogenic
- Likely-pathogenic
- Uncertain (VUS)
- Likely-benign
- Benign

# Let's guess the evidences

# Family pedigree

## Classic *BRCA1* Pedigree



# Segregation data (BS1, PP1)

- Caveat: linkage disequilibrium
- Penetration rate
- Difficult statistical evaluation

# Population data

2016

Medical and Population  
Genetics Primer

ExAC: mutational  
constraint and de  
novo mutations



Kaitlin Samocha

Broad Institute;  
Massachusetts General Hospital



# Population data

- 5%: benign stand alone (BA1)
- 0.5-5% (BS1)
- Wow! The first time observed variant!  
(Absent in population DB, PM2)

# Null variant

- Frameshift, Nonsense, canonical +-1 or 2 splicing site, initiation codon
- Caveat: LOF variants at the extreme 3' end of a gene
- Caveat: presence of multiple transcripts

# Computational (in silico) data

- PolyPhen2, SIFT, MutationTaster, etc
- Mutational hot spot and/or critical and well-established (PM1)
- Protein length changes due to in-frame deletions/insertions and stop losses functional domain (PM4 BP3)
- Novel missense at the same position (PM5)

# Other evidence

- de novo variants (PS2 PM6)
- functional studies (PS3 BS3)
- Allelic data (BP2 PM3)

# Evidences of interpretation

- Population data
- Computational data
- Functional data
- Segregation data
- De novo data
- Allele data
- Other databases
- Other data

# 27 variant attributes

	Benign			Pathogenic		
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
<b>Population data</b>	MAF is too high for disorder BA1/BS1 OR observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
<b>Computational and predictive data</b>		Multiple lines of computational evidence suggest no impact on gene /gene product BP4  Missense in gene where only truncating cause disease BP1  Silent variant with non predicted splice impact BP7  In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5  Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
<b>Functional data</b>	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
<b>Segregation data</b>	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data		
<b>De novo data</b>				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
<b>Allelic data</b>		Observed in <i>trans</i> with a dominant variant BP2  Observed in <i>cis</i> with a pathogenic variant BP2		For recessive disorders, detected in <i>trans</i> with a pathogenic variant PM3		
<b>Other database</b>		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
<b>Other data</b>		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

# Rules for combining criteria to classify sequence variants

Pathogenic	(i) 1 Very strong (PVS1) AND (a) $\geq 1$ Strong (PS1–PS4) OR (b) $\geq 2$ Moderate (PM1–PM6) OR (c) 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) OR (d) $\geq 2$ Supporting (PP1–PP5) (ii) $\geq 2$ Strong (PS1–PS4) OR (iii) 1 Strong (PS1–PS4) AND (a) $\geq 3$ Moderate (PM1–PM6) OR (b) 2 Moderate (PM1–PM6) AND $\geq 2$ Supporting (PP1–PP5) OR (c) 1 Moderate (PM1–PM6) AND $\geq 4$ supporting (PP1–PP5)
Likely pathogenic	(i) 1 Very strong (PVS1) AND 1 moderate (PM1–PM6) OR (ii) 1 Strong (PS1–PS4) AND 1–2 moderate (PM1–PM6) OR (iii) 1 Strong (PS1–PS4) AND $\geq 2$ supporting (PP1–PP5) OR (iv) $\geq 3$ Moderate (PM1–PM6) OR (v) 2 Moderate (PM1–PM6) AND $\geq 2$ supporting (PP1–PP5) OR (vi) 1 Moderate (PM1–PM6) AND $\geq 4$ supporting (PP1–PP5)
Benign	(i) 1 Stand-alone (BA1) OR (ii) $\geq 2$ Strong (BS1–BS4)
Likely benign	(i) 1 Strong (BS1–BS4) and 1 supporting (BP1–BP7) OR (ii) $\geq 2$ Supporting (BP1–BP7)
Uncertain significance	(i) Other criteria shown above are not met OR (ii) the criteria for benign and pathogenic are contradictory

## **Evaluation of ACMG-Guideline-Based Variant Classification of Cancer Susceptibility and Non-Cancer-Associated Genes in Families Affected by Breast Cancer [2]**

- Whole-exome sequencing
- 180 medically relevant genes
- 404 individuals
- 253 families
- 1,640 variants

# Example (VUS)

- 76/M, Lung adenocarcinoma
- NM\_000059.3(BRCA2):c.5683G>A  
(p.Glu1895Lys)

**Which evidences among 27 variants  
attributes are available in our lab?**

Ion torrent

# Characteristics of BRCA1/2

- LOF known mechanism of disease (for PVS1)
- Mode of inheritance (for PM3/BP2)
  - AD/AR (BRCA2)
- Missense pathogenic (for PP2/BP1)
  - BRCA2 1%
- Hot spot or critical/well-established functional domain (for PM1)
  - BRCA2, Helical (2479-2667), OB (2670-2799 and 3052-3190), Tower (2831-2872)

# NM\_000059.3(BRCA2):c.5683G>A (p.Glu1895Lys)

- LOF known mechanism of disease (for PVS1)
- Mode of inheritance (for PM3/BP2)
  - AD/AR (BRCA2)
- Missense pathogenic (for PP2/BP1)
  - **BRCA2 1%**
- Hot spot or critical/well-established functional domain (for PM1)
  - BRCA2, Helical (2479-2667), OB (2670-2799 and 3052-3190), Tower (2831-2872)

# Characteristics of variant (NM\_000059.3(BRCA2):c.5683G>A (p.Glu1895Lys))

- ClinVar (Uncertain significance (Last evaluated: Nov 1, 2015)) (PP5, BP6)
- Population AF:  $8.29e^{-06}$  (PM2, BA1, BS1)
- Insilico SIFT 1.0, phyloP -0.72, PolyPhen-2 0.004 BP4

# Functional assay (BS3)

- Findlay (2018) Nature 562: 217 PubMed: 30209399
- Guidugli (2013) Cancer Res 73: 265 PubMed: 23108138
- Biswas (2011) Blood 118: 2430 PubMed: 21719596
- Becker (2012) Breast Cancer Res Treat 135: 167 PubMed: 22729890

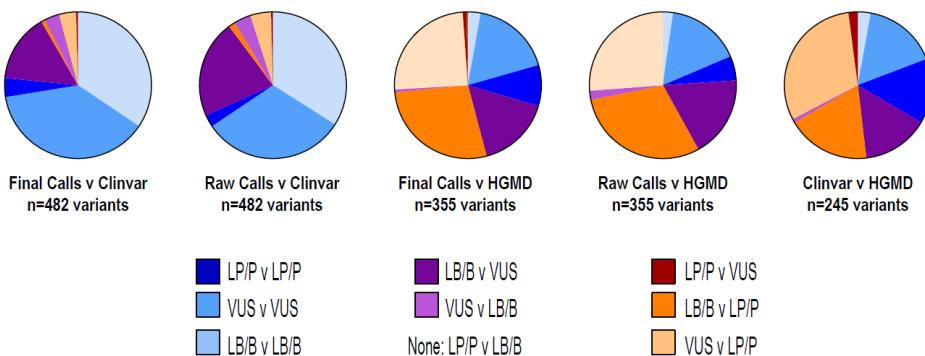
# Summary

- **Likely benign**
- $\geq 2$  supporting (BP1, BP4)

# In Maxwell study [2]

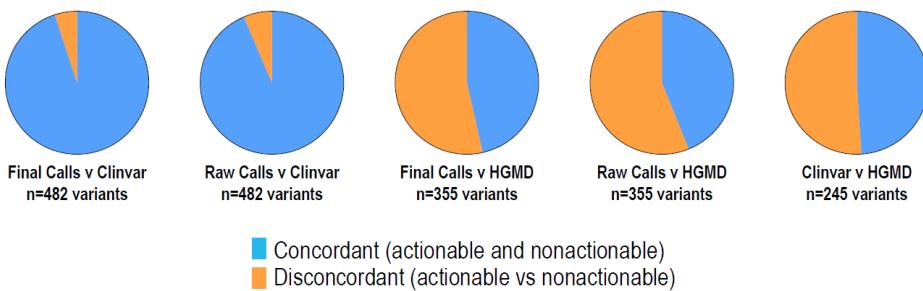
A.

Call concordance between ACMG, Clinvar and HGMD calls



B.

Potential actionability concordance between ACMG, Clinvar and HGMD calls



# Question

- Can our lab classify novel missense variant into pathogenic or benign, not VUS?

# Answer

- Yes, we can but...

# Unrobust result

- BP4 (How many in silico test)

# Experience

# **Sherloc: a comprehensive refinement of the ACMG–AMP variant classification criteria [3]**

- Iterative refinement of ACMG guideline
- 33 ACMG criteria to 108 criteria
- Combination of evidence to  
semiquantitative system

# CMC pathology lab

- In house database
- <https://brcaexchange.org/>
- <http://coda.nih.go.kr/coda/KRGDB/>
- <https://www.ncbi.nlm.nih.gov/clinvar/>
- <https://gnomad.broadinstitute.org/>

**BRCA2 c.10150C>T p.Arfs3384Ter**

# KOBRA [4]

Breast Cancer Res Treat (2015) 151:157–168  
DOI 10.1007/s10549-015-3377-4



## EPIDEMIOLOGY

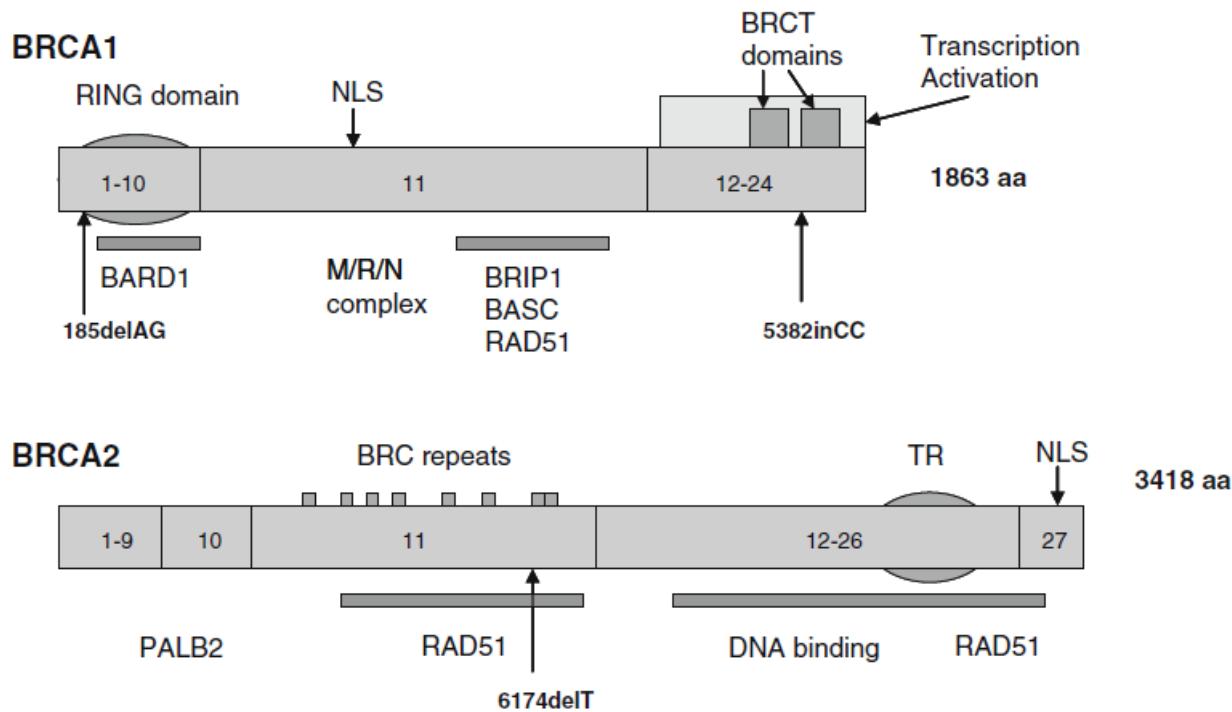
### The prevalence and spectrum of *BRCA1* and *BRCA2* mutations in Korean population: recent update of the Korean Hereditary Breast Cancer (KOHBRA) study

Eunyoung Kang<sup>1</sup> · Moon-Woo Seong<sup>2</sup> · Sue K. Park<sup>3,4,5</sup> ·  
Jong Won Lee<sup>6</sup> · Jihyoun Lee<sup>7</sup> · Lee Su Kim<sup>8</sup> · Jeong Eon Lee<sup>9</sup> ·  
Sung Yong Kim<sup>10</sup> · Joon Jeong<sup>11</sup> · Sang Ah Han<sup>12</sup> · Sung-Won Kim<sup>1</sup> ·  
Korean Hereditary Breast Cancer Study Group

Received: 30 January 2015 / Accepted: 7 April 2015 / Published online: 12 April 2015  
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25	9503del4	c.9275_9278delATTT	p.Tyr3092Cysfs	1	2
25	9668insC	c.9440_9441insC	p.Ala3148Cysfs	Novel	1
27	10378C>T	c.10150C>T	p.Arg3384Ter	1	1
27	9927del4	c.9699_9702delTATG	p.Cys3233Trpfs	3	1

# End truncation



# **HUMAN MUTATION Mutation in Brief 31: E1200-E1240 (2010) Online**

Finally, three sequence variants – BRCA2 c.9976A>T (BIC: K3326X), c.10095delCins11 (BIC: 10323delCins11) and c.10150C>T (BIC: R3384X) predicted to result in protein truncation were ruled as exceptions that **could not be classified** because of their **location near the 3'-end** and possibly dispensable part of the gene.

# Clinvar

BRCA2 c.9976A>T (p.Lys3326\*) variant,  
located upstream of this variant and also in the  
last exon of the gene, is a known benign  
variant.

# Conclusions

- ACMG guideline
- Systemaic review system is required
- Let's determine VUS to benign or even pathogenic

# References

- [1] S. Richards, N. Aziz, S. Bale, et al. "Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology". En. In: *Genetics in Medicine* 17.5 (5. 2015), pp. 405-423. ISSN: 1530-0366.
- [2] K. Maxwell, S. Hart, J. Vijai, et al. "Evaluation of ACMG-Guideline-Based Variant Classification of Cancer Susceptibility and Non-Cancer-Associated Genes in Families Affected by Breast Cancer". In: *The American Journal of Human Genetics* 98.5 (5. 2016), pp. 801-817. ISSN: 0002-9297.
- [3] K. Nykamp, M. Anderson, M. Powers, et al. "Sherloc: a comprehensive refinement of the ACMG<93>AMP variant classification criteria". En. In: *Genet Med* 19.10 (10. 2017), pp. 1105-1117. ISSN: 1530-0366.
- [4] E. Kang, M. Seong, S. K. Park, et al. "The prevalence and spectrum of BRCA1 and BRCA2 mutations in Korean population: recent update of the Korean Hereditary Breast Cancer (KOHBRA) study". En. In: *Breast Cancer Res Treat* 151.1 (5. 2015), pp. 157-168. ISSN: 1573-7217.