Reduced Risk *BRCA1* and *BRCA2* variants: Insight into Classification of Concordant Variants Between Two Commercial Laboratories

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INTRODUCTION

- Identification of reduced risk *BRCA1* and *BRCA2* (RR-*BRCA*) variants is often challenging because many classification models are designed for typical risk Mendelian variants.
- RR-*BRCA* risks need to be determined by statistical models requiring substantial amounts of data.
- Reporting of RR-*BRCA* variants is often inconsistent across laboratories due to lack of consensus and terminology.
- Counseling patients with RR-*BRCA* variants is complex and there are currently no guidelines.

METHODS

- A list of RR-*BRCA* variants were compiled by two large US genetics clinical diagnostic laboratories.
- Rationale supporting a RR-*BRCA* interpretation were provided.
- Unpublished and publicly available clinical, functional, population, and predictive data were collected.

RESULTS

- Laboratories had different but complementary approaches in identifying RR-*BRCA* variants including the consideration of the following:
  - the identification of biallelic Fanconi Anemia-affected patients
  - variant type (missense, spliceogenic, etc.)
  - alternative splicing
  - identification of NMD-escaping events
  - laboratory-validated cancer history weighting models
  - published reduced risk data
  - extrapolation of a reduced-risk interpretation onto close match variants that are expected to have the same effect.
- 30 variants were observed by both laboratories and considered by at least one as RR-*BRCA*:
  - 13 variants were considered RR-*BRCA* by both laboratories
    - *BRCA1:* included c.5096G>A (p.R1699Q) and variants impacting the canonical c.671 splice acceptor site
    - *BRCA2:* included three frameshift (c.658_658delGT, c.9672dupA, c.9699_9702delTATG); two spliceogenic (c.8488-1G>A and c.8488-1G>T); and two missense [c.7878G>C (p.W2626C), c.9302T>G (p.L3101R) variants.

CONCLUSIONS

- Despite different but complementary interpretation strategies across two laboratories, consistent results were obtained for 13 RR-*BRCA* variants providing evidence for a less severe phenotype.
- Consequently, these variants may require less stringent management strategies compared to traditional pathogenic *BRCA* variants depending on individual and family history.
- Standardized reporting will be of great benefit for patients and care teams.
- Further work to define risk thresholds and categories for reporting RR-*BRCA* variants will be of great clinical value to personalize cancer risks in conjunction with other clinical and genetic risk factors, including polygenic risk scores.
- Opportunities to harmonize variant interpretation and standardized reporting will be of great benefit for patients and care teams.

TABLES AND FIGURES

Table 1. List of concordantly classified reduced risk BRCA1 and BRCA2 variants

	DNA Nucleotide Change (Alias, in brackets, if applicable)	Variant Type	gnomAD v2.1.1 Filtering Allele Freq (FAF)	gnomAD v2.1.1 highest Minor Allele Freq (MAF)	Splicing prediction SpliceAI-window 5000bp Acceptor Loss (AL); Donor Loss (DL); Acceptor Gain (AG); Donor Gain (DG). Scores <.20 are considered inconsequential	ACMG evidence criteria derived from functional data
BRCA1	c.5096G>A (p.R1699Q)	Missense	.0000229 (NFE)	.00005281 (NFE)	Inconsequential	PS3
	c.671-2A>G	Splice	Absent	Absent	AL: 0.98; DL: 0.28 to 0.30; AG & DG: inconsequential Prediction: r.671_4096del (p.A224_L1365del)	N/A
	c.671-2A>C	Splice	N/A	.00006898 (AFR)		N/A
	c.671-1G>T	Splice	Absent	Absent		N/A
	c.671-1G>C	Splice	Absent	Absent		N/A
	c.671-1G>A	Splice	Absent	Absent		N/A
BRCA2	c.658_659DELGT	Frameshift	0.000029 (NFE)	.0001262 (AFR)	Inconsequential	N/A
	c.9672DUPA	Frameshift	N/A	.00000883 (NFE)	Inconsequential	N/A
	c.9699_9702DELTATG	Frameshift	.0002461 (LAT)	.0003974 (LAT)	Inconsequential	N/A
	c.7878G>C (p.W2626C)	Missense	.0000029 (NFE)	.00001761 (NFE)	Inconsequential	PS3
	c.9302T>G (p.L3101R)	Missense	Absent	Absent	Inconsequential	PS3
	c.8488-1G>A	Splice	Absent	Absent	AL: 0.92; AG: 0.37 to 0.49; DL & DG: inconsequential Prediction 1: r.8488_8632del (p.W2830Kfs*13) Prediction 2: r.8488_8499del (p.W2830_K2833del)	N/A
	c.8488-1G>T	Splice	Absent	Absent		N/A

Variants observed and identified by only one laboratory as possible RR-*BRCA*

BRCA1:

- **Truncating**: c.1292T>G (p.L431*) |c.2706_2707dupAT

BRCA2:

- **Truncating**: c.1310_1313DELAAGA | c.4284dupT | c.5303_5304delTT
- **Splicing**: c.517-2A>G| c.631 donor site (N=3 variants) | c.67+3A>G | c.7007G>A last-nucleotide (N=2 variants) | c.8487+3A>G
- **Missense**: c.7529T>C (p.L2510P) | c.7964A>G (p.W2655R) | c.8009C>T (p.S2670L) | c.8524C>T (p.R2842C)

Figure 2: Functional Data for Concordant RR-*BRCA* Variants

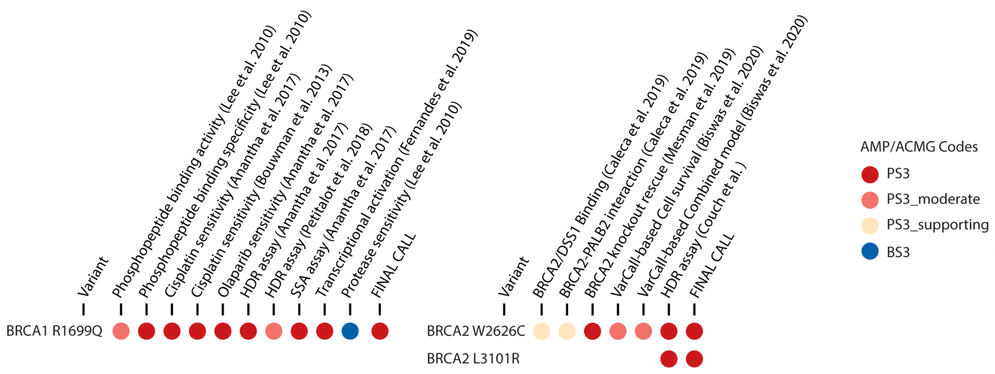

Figure 2. Available literature-based and unpublished cumulative functional evidence for three variants were compiled and coded per ACMG-AMP codes (PS3 functional evidence towards pathogenic; BS3 functional evidence towards benign) and strength (down-weighted from baseline 'strong' to either 'moderate' or 'supporting' where denoted) as approved by the ClinGen BRCA1/2 Variant Curation Expert Panel (Lyra *et al* 2021). A final call on functional strength is provided based on the cumulative functional evidence.

Figure 1: Example Family History Curves for Reduced Risk Variants

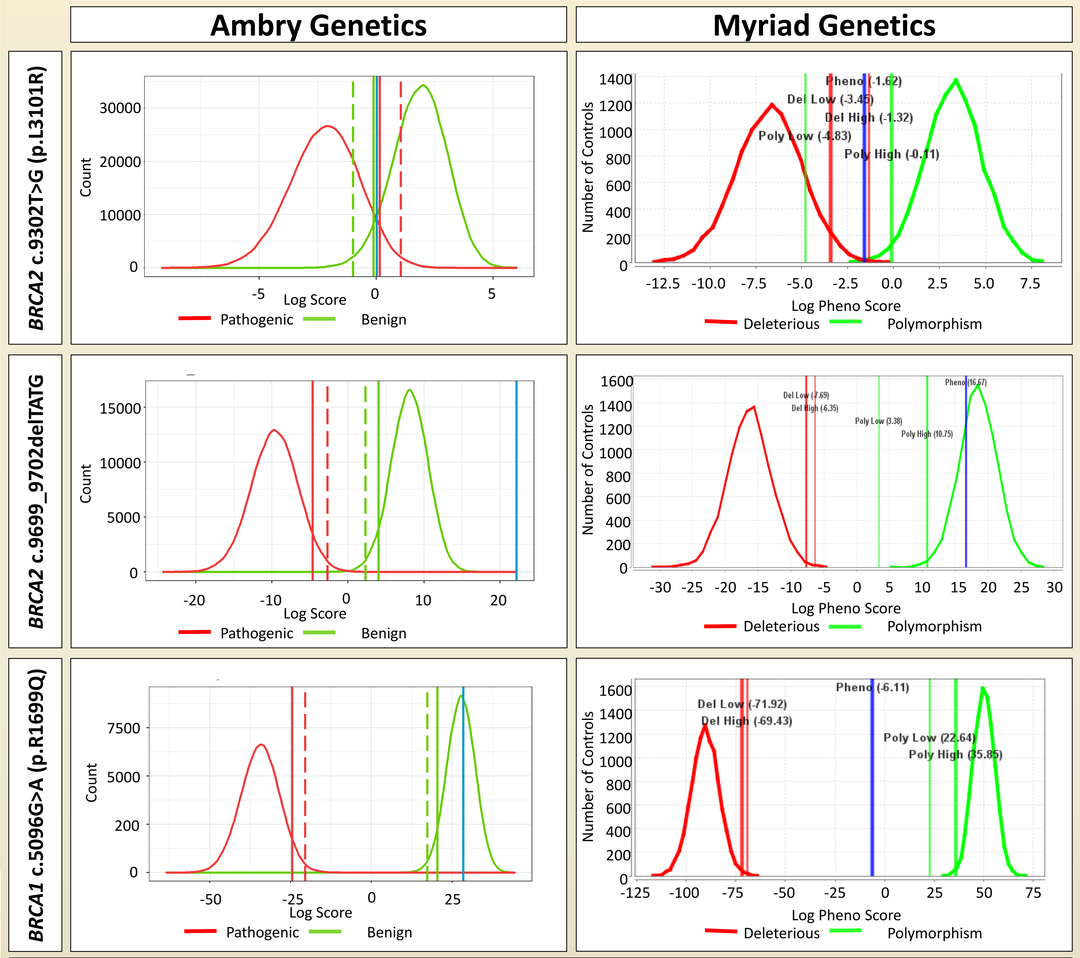

Figure 1. Clinical history curves for three representative variants are provided. Aggregate clinical data of variant carriers (blue line) is plotted relative to the distribution of carriers of known pathogenic (red) and known benign (green) variants. Solid and dashed lines represent the conservative 95th and 99th percentile confidence bounds for pathogenic and benign curves. Pruss *et al.* 2014, Li *et al.* 2020.

REFERENCES

Anantha, R.W. *et al.* Functional and mutational landscapes of BRCA1 for homology-directed repair and therapy resistance. *Elife* **6**, pii: e21350 (2017).

Biswas, K. *et al.* A computational model for classification of BRCA2 variants using mouse embryonic stem cell-based functional assays. *NPJ Genom Med* **5**, 52 (2020).

Bouwman, P. *et al.* A high-throughput functional complementation assay for classification of BRCA1 missense variants. *Cancer Discov* **3**, 1142-55 (2013).

Caleca, L. *et al.* GFP-Fragment Reassembly Screens for the Functional Characterization of Variants of Uncertain Significance in Protein Interaction Domains of the BRCA1 and BRCA2 Genes. *Cancers (Basel)* **11**(2019).

Fernandes, V.C. *et al.* Impact of amino acid substitutions at secondary structures in the BRCT domains of the tumor suppressor BRCA1: Implications for clinical annotation. *J Biol Chem* **294**, 5980-5992 (2019).

Lee, M.S. *et al.* Comprehensive analysis of missense variations in the BRCT domain of BRCA1 by structural and functional assays. *Cancer Res* **70**, 4880-90 (2010).

Li, H. *et al.* Classification of variants of uncertain significance in BRCA1 and BRCA2 using personal and family history of cancer from individuals in a large hereditary cancer multigene panel testing cohort. *Genet Med.* **22**, 701-708 (2020).

Lyra *et al*. Integration of functional assay data results provides strong evidence for classification of hundreds of BRCA1 variants of uncertain significance. *Genet Med.* **23**, 306-315 (2021).

Mesman, R.L.S. *et al.* The functional impact of variants of uncertain significance in BRCA2. *Genet Med* **21**, 293-302 (2019).

Petitalot, A. *et al.* Combining Homologous Recombination and Phosphopeptide-binding Data to Predict the Impact of BRCA1 BRCT Variants on Cancer Risk. *Mol Cancer Res* **17**, 54-69 (2019).

Pruss, D. *et al.* Development and validation of a new algorithm for the reclassification of genetic variants identified in the BRCA1 and BRCA2 genes. *Breast Cancer Res Treat.* **147**, 119-32 (2014).