Integration of Protein Stability and Structural Context Scores Improves Bioinformatics Predictions for BRCA1 and TP53 Gene Variants

N. Rotenberg[1,2], L. Ramadane-Morchadi[4], MJ. Varga[3], A. Chamberlin[3], ME. Richardson[3], C. Fortuno[1], M. de la Hoya[4], AB. Spurdle[1,2]

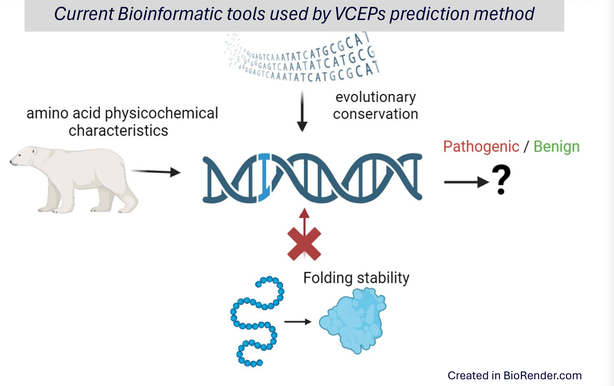
1. Molecular Cancer Epidemiology, QIMR Berghofer MRI, Australia
2. University of Queensland, QLD, Australia.
3. Ambry Genetics, Aliso Viejo, CA, USA.
4. Molecular Oncology Laboratory, IdISSC, Madrid, Spain.

Queensland Institute of Medical Research
The University Of Queensland AUSTRALIA
www.qimr.edu.au
IdISSC Instituto de Investigación Sanitaria Hospital Clínico San Carlos
Hospital Clínico San Carlos SaludMadrid

BACKGROUND

The clinical classification of genetic variants encoding missense variants and single amino acid deletions is especially challenging. The general American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) guidelines, and in particular specifications of these guidelines developed by Variant Curation Expert Panels (VCEPs) for particular genes, have helped decrease the number of variants of uncertain clinical significance (VUS). Nevertheless, a **high proportion of missense and in-frame deletions remain as VUS or have conflicting evidence in ClinVar. One mechanism in which a variant can result in loss of function of the protein is a reduction in thermodynamic stability (1).** The tolerance to a change in the stability of the protein can alter depending on the location of the variant (2). Changes in folding stability can be captured using with computer algorithms by calculating Gibbs Free Energy scores (ΔΔG). An alternative is AlphaMissense, a deep learning tool recently developed by Google DeepMind, utilises structural context to predict pathogenicity for all human proteome missense variants (3). **Bioinformatics tools currently in use by VCEPs do not capture ΔΔG changes and do not include AlphaMissense.** Our study aimed to investigate whether structure-based prediction methods outperform current bioinformatics tools in discriminating pathogenic and benign *BRCA1* and *TP53* variants.

Current Bioinformatic tools used by VCEPs prediction method



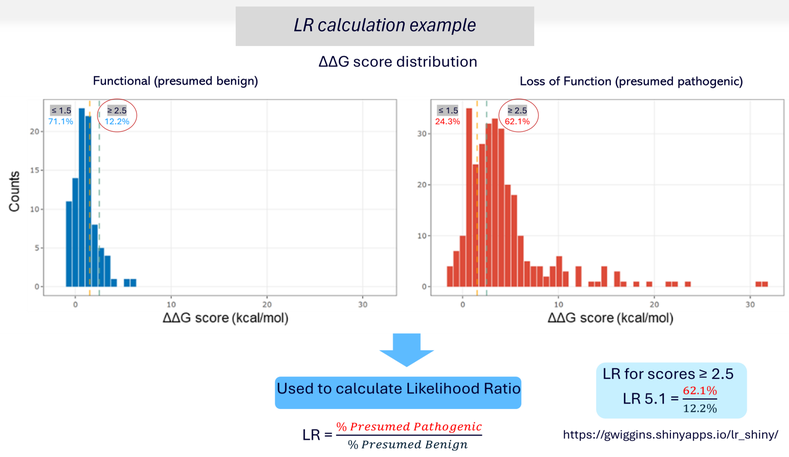
METHODS

We focussed our analysis on missense variants in BRCA1 and p53, and single amino acid deletions in p53, only. ΔΔG values were predicted with FoldX 5.0 (missense variants), and AlphaFold2/RosettaRelax protocol of Woods *et al*. 2023 (deletions) (4). For p53 missense variants, only experimental PDBs produced by X-ray crystallography were utilised as input for ΔΔG prediction; however, for BRCA1 we used experimental PDBs produced by NMR (RING domain) or X-ray crystallography (BRCT domain), and also structure models produced by **AlphaFold2. Relative Solvent Accessibility (RSA)** was computed for every amino acid residue to differentiate between Surface, p.Buried, and Buried residues. Additionally, **IUPred scores** were produced to determine the residue's disorder tendency. Highly disordered residues were excluded from further analysis. **AlphaMissense** scores were retrieved at *console.cloud.google.com/storage/browser/dm_alphamissense*.

Reference variant sets were compiled from three different functional datasets for p53 variants**; Giacomelli et al. 2018 (5), Kato et at. 2003 (6)** for missense variants **and Kotler et al. 2018 (7**) for single amino acid deletions. Score ranges as indicated in the original report were used to define variant impact on function, used as a surrogate to categorise each variant as presumed pathogenic or presumed benign. For BRCA1, the **MAVE dataset (8)** was used as a reference set. The ΔΔG score range categories (cut-off scores) that best predicted pathogenicity were determined using an online tool set up to simplify and compare likelihood ratio (LR) calculations for bioinformatic prediction tools (*https://gwiggins.shinyapps.io/lr_shiny*). Optimal ΔΔG cut-off scores were chosen by reviewing the ΔΔG distribution of reference set variants within each RSA category and altering the cutpoints in a sequential process to maximise the number of variants assigned evidence weight based on estimated LR. **Performance** of ΔΔG, AlphaMissense and two broadly accepted computational tools (BayesDel and Align-GVGD categories used by the TP53 VCEP), **was evaluated using auROC, Boruta and Binary logistic regression.**

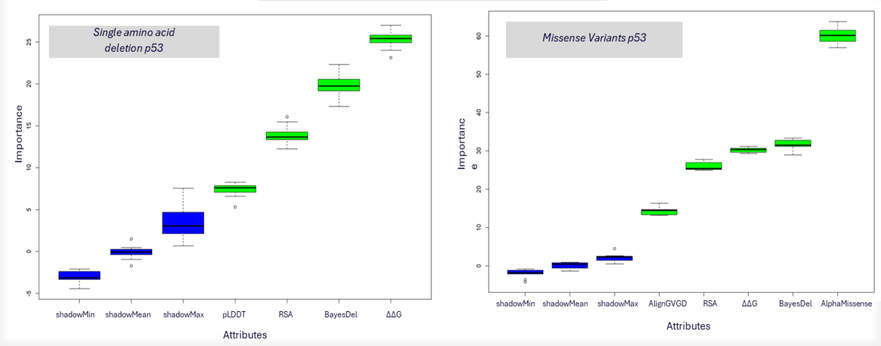
Estimated LRs towards pathogenicity, using the defined reference sets, were used to **define categories that best predict pathogenicity for both individual and combined predictors.** To transform LRs into evidence strengths, we followed recommendations arising from Bayesian modelling of the ACMG/AMP criteria (9). For BRCA1, data from the **BRIDGES breast cancer case-control sequencing study (10)** was used as a Case-control Validation Dataset and used to perform a burden analysis clinical validation of major findings.

LR calculation example



RESULTS

Boruta feature selection analysis



p53 Missense variants:

Boruta feature selection and the binary logistic regression showed AlphaMissense to outperform other predictive tools. Logistic regression analysis indicated that Align-GVGD provided no significant predictive value after considering the other annotations.

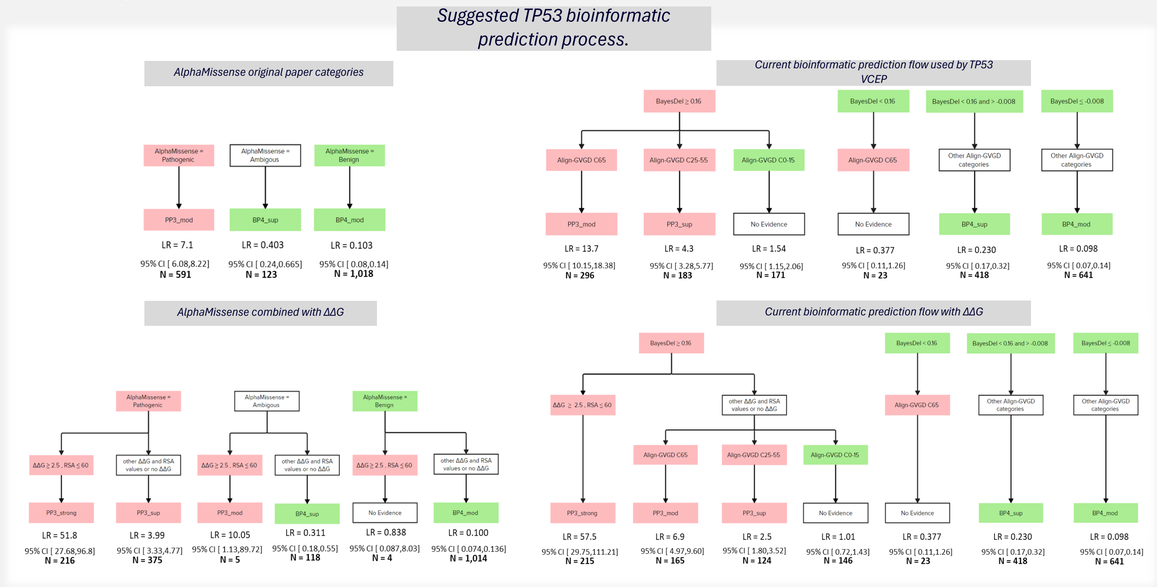
p53 single amino acid deletions:

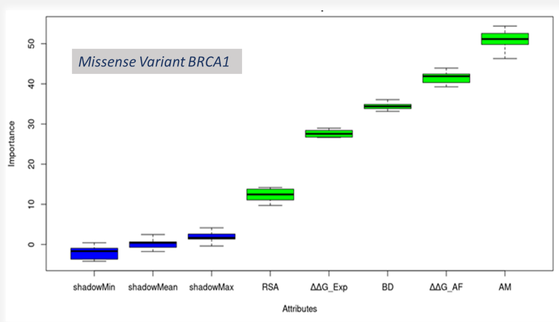
For single-amino acid deletion variant impact prediction, the Boruta feature selection analysis revealed ΔΔG to have the highest importance, followed by BayesDel, RSA and pLDDT. **ΔΔG ≥ 2.5 REU for buried residues (RSA ≤ 25%) outperform currently used BayesDel cut-off score by TP53 VCEP**, providing Moderate evidence towards pathogenicity (LR = 8.6 CI 95% [2.7, 24.4]) compared to No evidence for BayesDel ≥ 0.16 (LR = 1.2 CI 95% [1.0,1.3]).

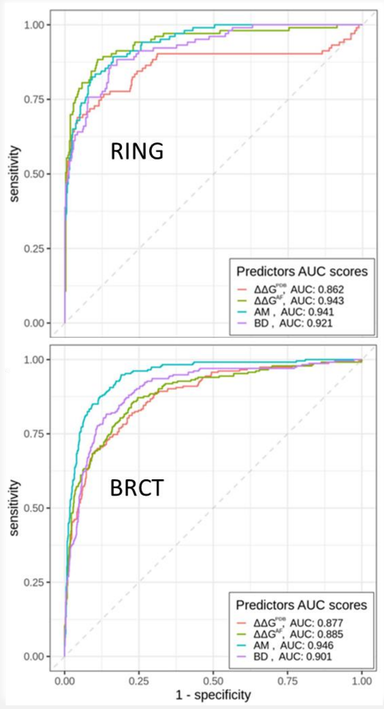
Based on these findings, we reassessed and created three potential flowcharts, as shown below.

Buried/p.Buried residues (Relative solvent accessibility ≤ 60%), ΔΔG pathogenicity thresholds (≤1.5/≥2.5kcal/mol) improved currently used prediction approaches for missense variants. Combining ΔΔG with the pre-specified AlphaMissense categories had the highest specificity (0.894) compared to other models tested (0.861-0.884)

Suggested TP53 bioinformatic prediction process.







BRCA1 Missense variants

Above: Boruta feature selection and the binary logistic regression showed AlphaMissense (AM) outperforming ΔΔG, BayesDel (BD) and RSA. Interestingly, AlphaFold2-based ΔΔG predictions ($\Delta\Delta G^{AF}$) outperform experimental structure-based predictions ($\Delta\Delta G^{PDB}$), albeit this effect is restricted exclusively to the RING domain.

Right: Analysis of AM, $\Delta\Delta G^{PDB}$, $\Delta\Delta G^{AF}$, and BD performance at discriminating LOF and FUNC at the RING and BRCT domains. For each predictor, a ROC plot and the corresponding auROC value are displayed. Overall, $\Delta\Delta G^{AF}$ provide the best discrimination at the RING domain, while AM provides the best discrimination at the BRCT domain. $\Delta\Delta G^{AF}$ outperforms $\Delta\Delta G^{PDB}$ at the RING domain (AUC 0.943 vs. 0.862) but performs similarly at the BRCT domain (AUC 0.885 vs 0.877).

TAKE HOME POINTS

- **AlphaMissense outperformed other bioinformatic prediction tools in use by *TP53* and ENIGMA *BRCA1/2* VCEPs**
- **Integrating AlphaMissense and ΔΔG improves computational predictions for BRCA1 and p53 missense variants**
- **Computational predictions perform better for variants targeting buried/partially buried (<60% RSA) residues**
- **ΔΔG scores ≥ 2.5 REU in buried residues outperformed currently used prediction approaches for p53 single amino acid deletions**
- **The BRCA1 analysis suggests that AlphaFold2 models might outperform NMR structures as templates for ΔΔG computational predictions**

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