STRUCTURAL BIOLOGY IN VARIANT CLASSIFICATION

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BACKGROUND

- Structural Biology is the study of the three-dimensional structure of proteins and nucleic acids.
- As the structure of these molecules is intimately associated with their function, mutations that impart structural changes may lead to the production of abnormal/malfunctioning proteins that cause disease.
- Consequently, the 2015 ACMG guidelines state that mutations located within a “critical and well-established functional domain” may be considered moderate evidence of pathogenicity.[1]
- This provides a platform for the use of Structural Biology in variant classification.
- In particular, this method is advantageous for missense variants where the current data, in isolation, are insufficient to lead to a definitive classification.
- While the methods underlying macromolecular structure determination are well established, the specialized training and required expertise have prevented structural analysis from being widely incorporated in the genetic testing field.

OBJECTIVE

- Here we show the benefits of incorporating Structural Biology, with other evidence, in variant classification in hereditary cancer testing.

METHODS

- Two variants of uncertain significance (VUS) were evaluated for potential reclassification: *BRCA1* c.5153G>C (p.Trp1718Ser) and *BRCA2* c.91T>A (p.Trp31Arg).
- Publicly available protein structures were downloaded from the Protein Data Bank (PDB),[2] validated using MolProbity[3] and PDB_REDO (PDB IDs 1T15 and 3EU7)[4] and analyzed in PyMOL.[5]
- Additional functional data in the published literature were gathered and analyzed.

RESULTS

CASE STUDY 1. *BRCA1* c.5153G>C (p.Trp1718Ser)

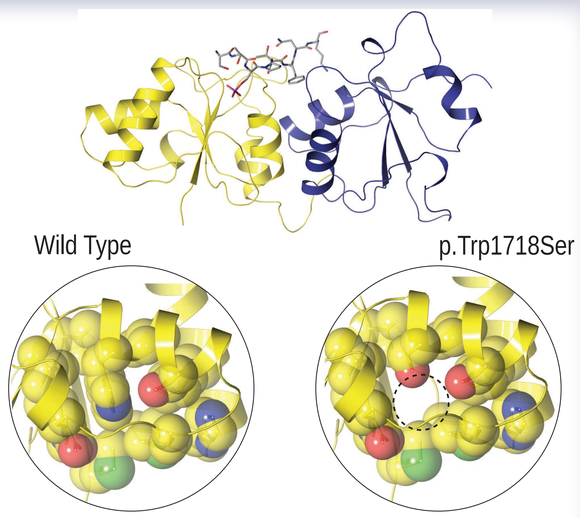
Wild Type Behavior

- The ability of the C-terminal BRCT domains to bind phosphorylated proteins is essential to the role of *BRCA1* as a tumor suppressor.[6]
- Trp1718 is buried in the core of the BRCT1 domain. Mutations buried in the protein core are frequently associated with disease.[7]

Impact of Variant

- The variant, Trp1718Ser, has a much smaller side chain creating a cavity in the protein core. Cavity creating mutations are known to be destabilizing to protein structure.
- In vitro, biochemical analysis of Trp1718Ser by Lee et al. confirms that the mutant protein harbors severe defects in protein folding and peptide binding, which compromises the downstream activity of *BRCA1*.[8]

Classification: Likely Pathogenic



CASE STUDY 2. *BRCA2* c.91T>A (p.Trp31Arg)

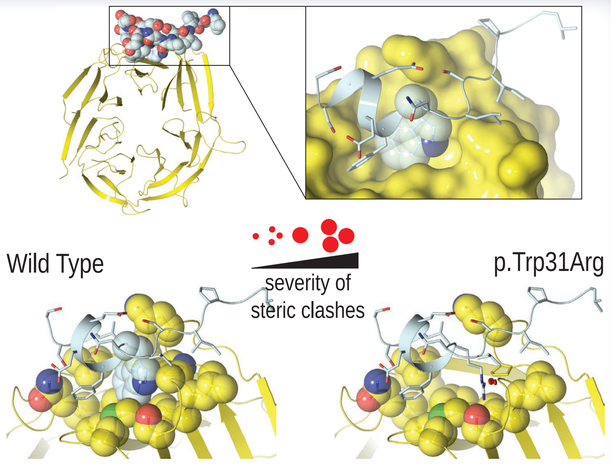
Wild Type Behavior

- The interaction of *BRCA2* with *PALB2* is essential to the function of the tumor suppressor protein in double-strand break repair (DSBR).[9]
- Trp31 lies in a pocket on the surface of the *PALB2* β-propeller domain.

Impact of Variant

- Arg fails to fill the pocket and also introduces steric clashes in the binding site with Gly1068 and Pro1097. The variant also buries the positive charge on the Arg guanidinium, which is left unbalanced by the local chemical environment. This makes the BRCA2•PALB2 interaction energetically unfavorable.
- Biswas et al. showed that mouse embryonic stem cells expressing Trp31Arg fail to rescue the conditional loss of *BRCA2*.[10] Xia et al. demonstrated that the variant failed to bind *BRCA2* and compromised DSBR repair similar to a negative control.[9]

Classification: Likely Pathogenic



- Structural analysis and functional evidence revealed that c.5153G>C (p.Trp1718Ser) severely impacts multiple functions of *BRCA1*, likely due to the introduction of a severe protein folding defect.
- This analysis also revealed that c.91T>A (p.Trp31Arg) abolishes a key interaction between *BRCA2* and *PALB2*.
- Based on this evidence, both mutations were reclassified from VUS to Likely Pathogenic.

CONCLUSION

- Structural Biology is a powerful tool in the reclassification of rare missense variants.
- This is demonstrated here, with two variants that we upgraded to Likely Pathogenic on the basis of protein structure analysis and functional data.

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