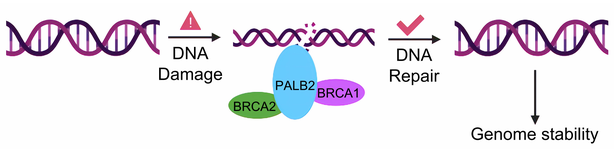
Investigating the Effects of Variants of Unknown Significance on the Binding Interaction between BRCA1 and PALB2 for Breast Cancer Predisposition

Precious Castillo, Davis E. Martin, and Mikaela D. Stewart. Texas Christian University, Fort Worth, TX

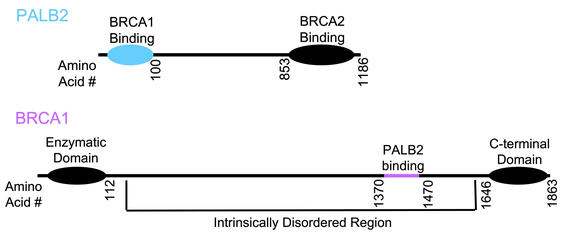
Introduction

BRCA1 and PALB2 interaction is essential in DNA damage repair.

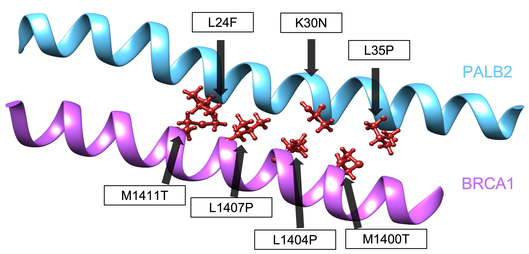


Above: Upon detection of DNA damage, PALB2 undergoes heterodimerization with BRCA1, leading to formation of the BRCA1-PALB2-BRCA2 complex which is then recruited to DNA double-strand break sites [1]. Failure to form this complex due to inherited mutations that result in a loss of function leads to obstructions in DNA damage repair and can increase breast cancer risk.

Predicted BRCA1 and PALB2 interaction region and the targeted Variants of Unknown Significance (VUS):



Above: Cartoons depicting the domain architecture of PALB2 and BRCA1. The regions important for heterodimerization are highlighted in blue and magenta respectively [2]. Ovals represent structured domains.

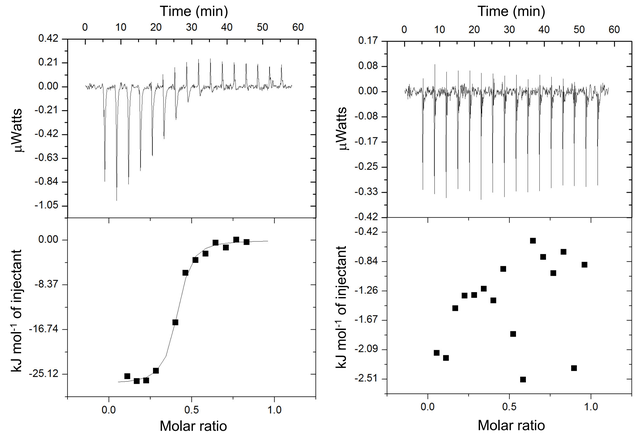


Above: Predicted heterodimeric coiled-coil interaction between BRCA1 and PALB2. Boxed texts show the different VUS that were created and assessed for their effects on the binding interaction. Each VUS was chosen from two databases (LOVD[3] and ClinVar) containing a list of mutations observed in patients with a family history of breast cancer. PDB ID 7K3S.

Methods / Objectives

- Recreate the BRCA1-PALB2 interaction with minimal constructs *in vitro*.
- Generate variants of unknown significance (VUS) within the predicted BRCA1-PALB2 binding domain via site-directed mutagenesis.
- Characterize chosen VUS using isothermal titration calorimetry (ITC) by measuring the enthalpic binding event between BRCA1 and PALB2.
- Optimize ITC as a tool for investigating the binding ability of natural variants associated with breast cancer in humans.

ITC data suggest that variants disrupt BRCA1-PALB2 binding interaction to varying degrees

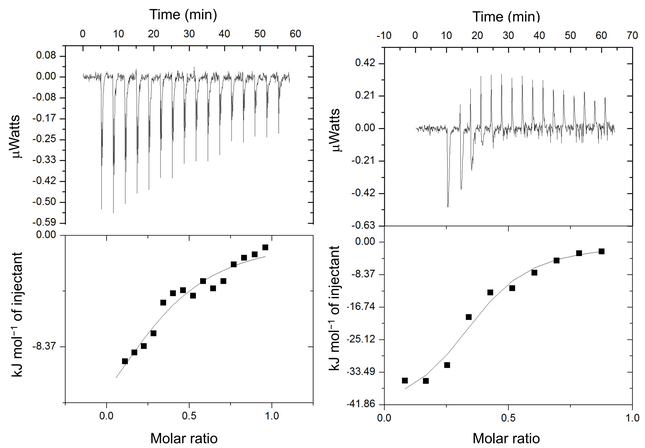


Normal binding interaction between BRCA1 and PALB2

L35P, L1404P, L1407P, M1411T completely abrogate binding

Left: ITC data from the titration of WT BRCA1 into WT PALB2 show that the proteins interact *in vitro* and the binding interaction is quantifiable by ITC. A dual endothermic/exothermic binding event is observed.

Right: Representative ITC data from the titration of BRCA1 L1404P into WT PALB2 showing complete disruption of BRCA1-PALB2 binding. Similar behavior was seen in PALB2 variant L35P and BRCA1 variants L1404P and M1411T.



M1400T shows altered binding mode

L24F and K30N show reduced binding affinity

Left: ITC data from the titration of M1400T BRCA1 into WT PALB2 show that the variant binds in an altered binding mode. Only an exothermic binding event is observed.

Right: Representative data from the titration of WT BRCA1 into PALB2 L24F showing similarities to the normal binding interaction but with reduced binding affinity. A dual endothermic/exothermic binding event is observed which is also seen in the normal binding interaction. Similar behavior was observed in PALB2 variant K30N.

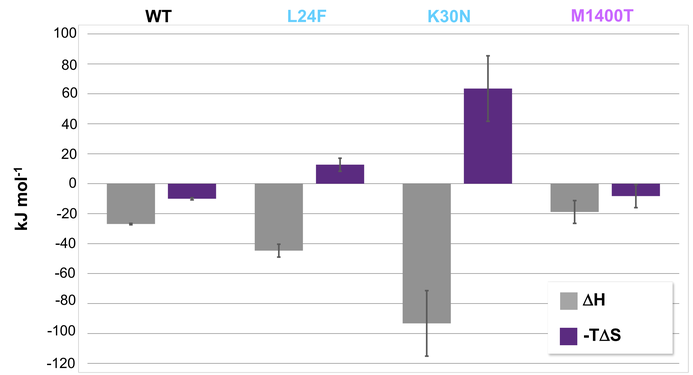
Variants show decreased binding affinity compared to WT BRCA1 and PALB2

	K_a (μM^{-1})	K_D (μM)
WT	2.98±0.68	0.336±0.076
L24F	0.40±0.14	2.51±0.92
K30N	0.166±0.080	6.02±2.89
M1400T	0.056±0.028	17.9±9.0

Left: Quantification of binding affinity from ITC single-site fit curves.

- WT PALB2 has the strongest binding affinity for WT BRCA1.
- PALB2 variants L24F and K30N, and BRCA1 variant M1400T show reduced binding affinity between BRCA1 and PALB2, but they do not completely abrogate binding.
- PALB2 L35P and BRCA1 L1404P, L1407P, and M1411T completely disrupt the binding interaction since no binding event was detected by ITC during the titration experiments.

Variants display changes in thermodynamic properties



Above: Quantification of the changes in enthalpy (**gray**) and entropy (**purple**) upon binding using ITC single-site fit curves. Error bars are derived from propagation of fitting error.

- PALB2 variants L24F and K30N exhibit a more negative enthalpy of binding compared to the WT, which might be indicative of the formation of new interactions in the protein complex. Both L24F and K30N exhibit a larger negative entropy value compared to the WT, which suggests that the entropy of the reaction was working against the success of the binding event.
- BRCA1 variant M1400T displays a less negative enthalpy of binding compared to the WT, suggesting a weakened interaction between BRCA1 and PALB2, which supports the idea of a less stable complex. M1400T has a positive entropy value similar to that of the WT.
- No data is shown for PALB2 L35P and BRCA1 M1411T, L1404P, and L1407P because no binding was detected by ITC, suggesting complete abrogation of the binding interaction.

Conclusions and Future Directions

An *in vitro* ITC assay can be used to investigate the function of BRCA1 and PALB2 variants. We evaluated several variants and their binding interactions by comparing them to the behavior of the WT.

- PALB2 variant L35P and BRCA1 variants M1411T, L1407P, and L1404P exhibit complete disruption of binding *in vitro,* consistent with previous cell-based studies that classified L35P, L1407P, and M1411T as deleterious to HR repair [3-4]. Our results suggest that these variants may be associated with increased breast cancer risk. In addition, we hypothesize that disruption of binding by the proline mutants might be due to proline's ability to act as an alpha helix breaker, and we are actively testing this hypothesis.

- BRCA1 M1400T appears to bind to WT PALB2, but the binding interaction is altered, which may indicate an altered cancer risk. PALB2 variants, L24F and K30N, show reduction in binding affinity to a lower degree relative to other variants, consistent with a previous cell-based study on K30N and its ability to bind to BRCA1 for HR [3]. Future studies should investigate these variants *in vivo* to determine if the change in binding interaction corresponds to a change in cellular function.

- Our future work will focus on collecting ITC data for other natural variants of unknown significance.

References and Funding

1. Sy, S.M.H., M.S.Y. Huen, and J.J. Chen, *PALB2 is an integral component of the BRCA complex required for homologous recombination repair.* Proceedings of the National Academy of Sciences of the United States of America, 2009. **106**(17): p. 7155-7160.
2. Zhang, F., et al., *PALB2 Links BRCA1 and BRCA2 in the DNA-Damage Response.* Current Biology, 2009. **19**(6): p. 524-529.
3. Foo, T.K., et al., *Compromised BRCA1-PALB2 interaction is associated with breast cancer risk.* Oncogene, 2017. **36**(29): p. 4161-4170.
4. Bouwman et al., *Functional categorization of BRCA1 variants of uncertain clinical significance in Homologous Recombination repair complementation assays.* Clinical Cancer Research, 2020. **26**(17): p.4559-4568.

Thank you to TCU College of Science and Engineering and NIH for funding (R15GM135900). Special thanks to TCU Chemistry Club for funding travel to Indianapolis.