Application of deep mutational scanning data for MLH1 variant interpretation

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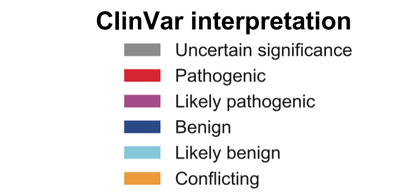
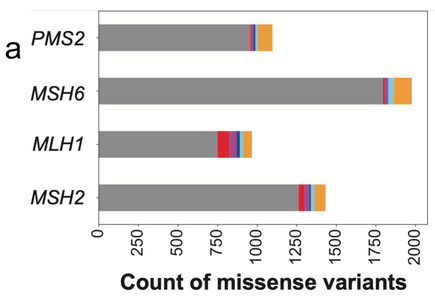
Introduction

- Lynch Syndrome (LS) is a cancer predisposition syndrome affecting more than 1 in every 300 individuals worldwide
- Tumorigenesis in LS is driven by germline loss-of-function variants in DNA mismatch repair (MMR) genes
- LS clinical genetic testing informs cancer surveillance but complicated by variants of uncertain significance (VUS) (Figure 1)
- Deep mutational scanning (DMS) measures effect of many variants simultaneously in a single assay with both basic science and clinical application, resulting in a "Loss-of-function" (LOF) Score to quantify their functional effect (Figure 1)
- These data have been previously shown to be successful for MSH2 variant reclassification, so we sought to apply *MLH1* DMS data to clinical datasets to determine their applicability





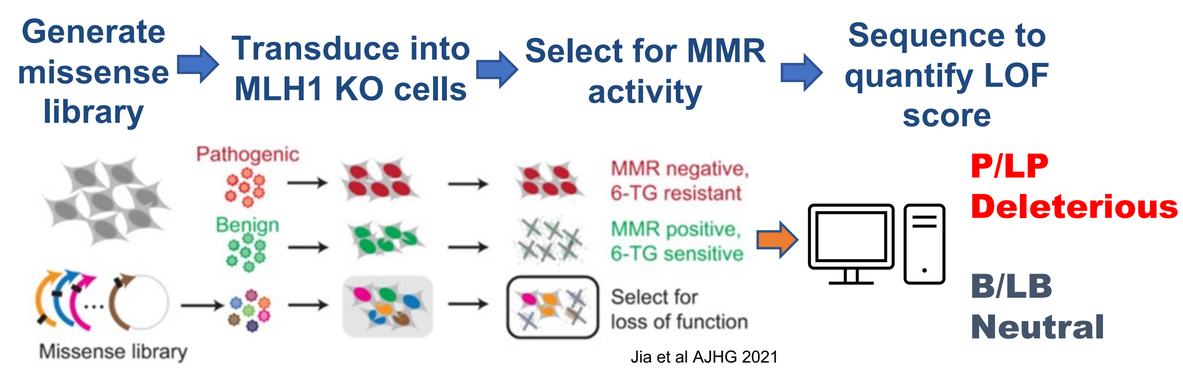


Figure 2: Diagram of the *MLH1* deep mutational scan. A MLH1 KO cell line is systematically transduced with a library containing nearly every possible missense MLH1 variant and then exposed to 6-thioguanine (6-TG) for selection of abrogated MMR activity; sequencing then allows quantification into an LOF score. *(Figure adapted from Jia et al, AJHG, 2021*).

Aim: Implement *MLH1* deep mutational scan data to improve clinical variant interpretation

Results – Validating LOF score for clinical use

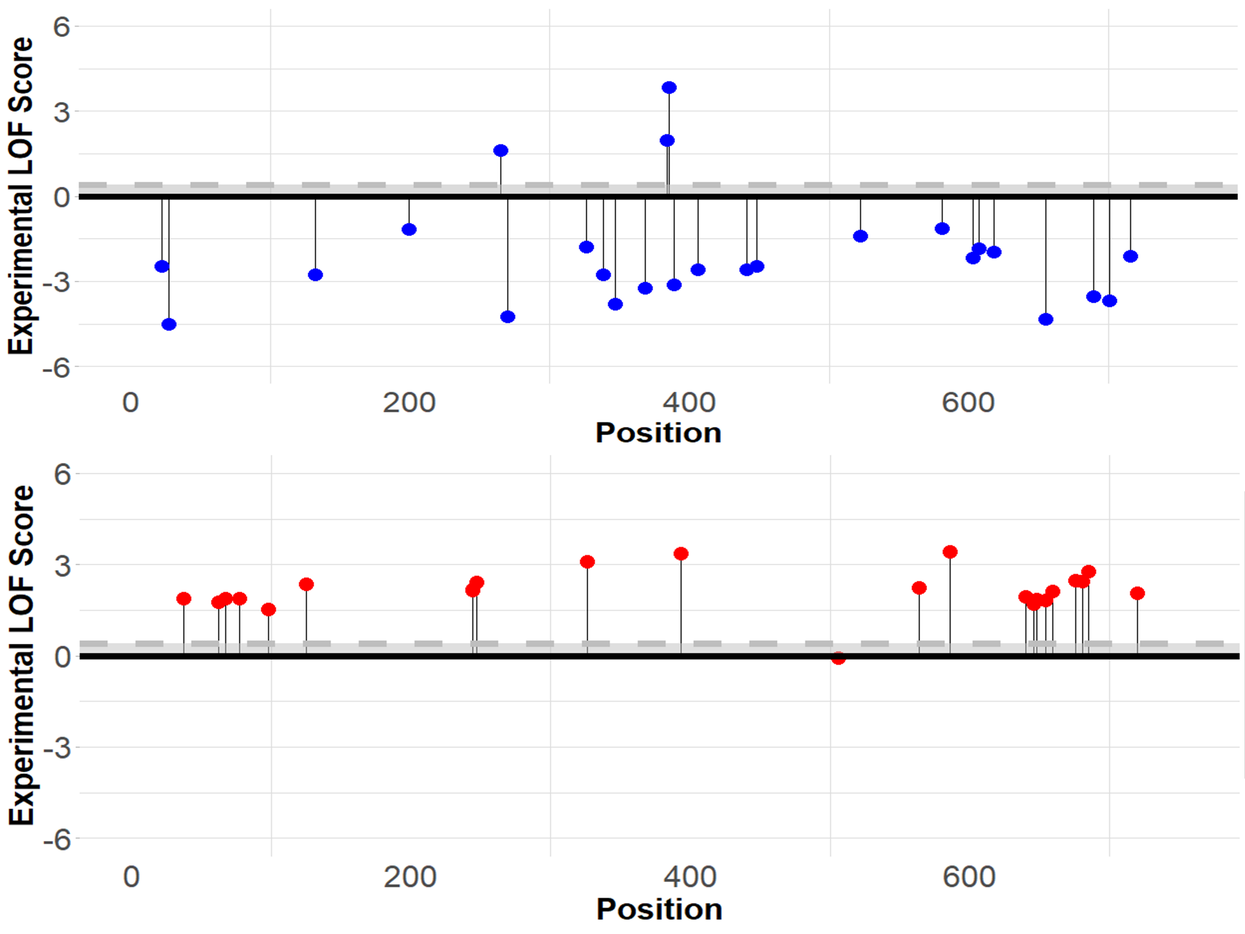


Figure 3: Lollipop plot of known pathogenic or benign variants. We overlaid the results of an *MLH1* DMS on clinical databases comprising >15,000 individuals with MMR gene variants from a clinical genetic testing laboratory. In order to determine their applicability to patients, we first applied these results to *MLH1* germline missense variants previously classified as Benign (N=27; top panel) or Pathogenic (N=23; bottom panel). All variants which exhibited neutral function in this screen had a benign classification, excluding one variant (c.1517T>C; p.V506A) for which the measured effect was intermediate. Conversely, most variants with abnormal function in our DMS data were previously classified as pathogenic or likely pathogenic, such that this function map provides strong evidence under the OddsPath framework (ClinGen Sequence Variant Interpretation Working Group, Tavtigian et al, *Genet Med* 2018).

Results – LOF Score distribution

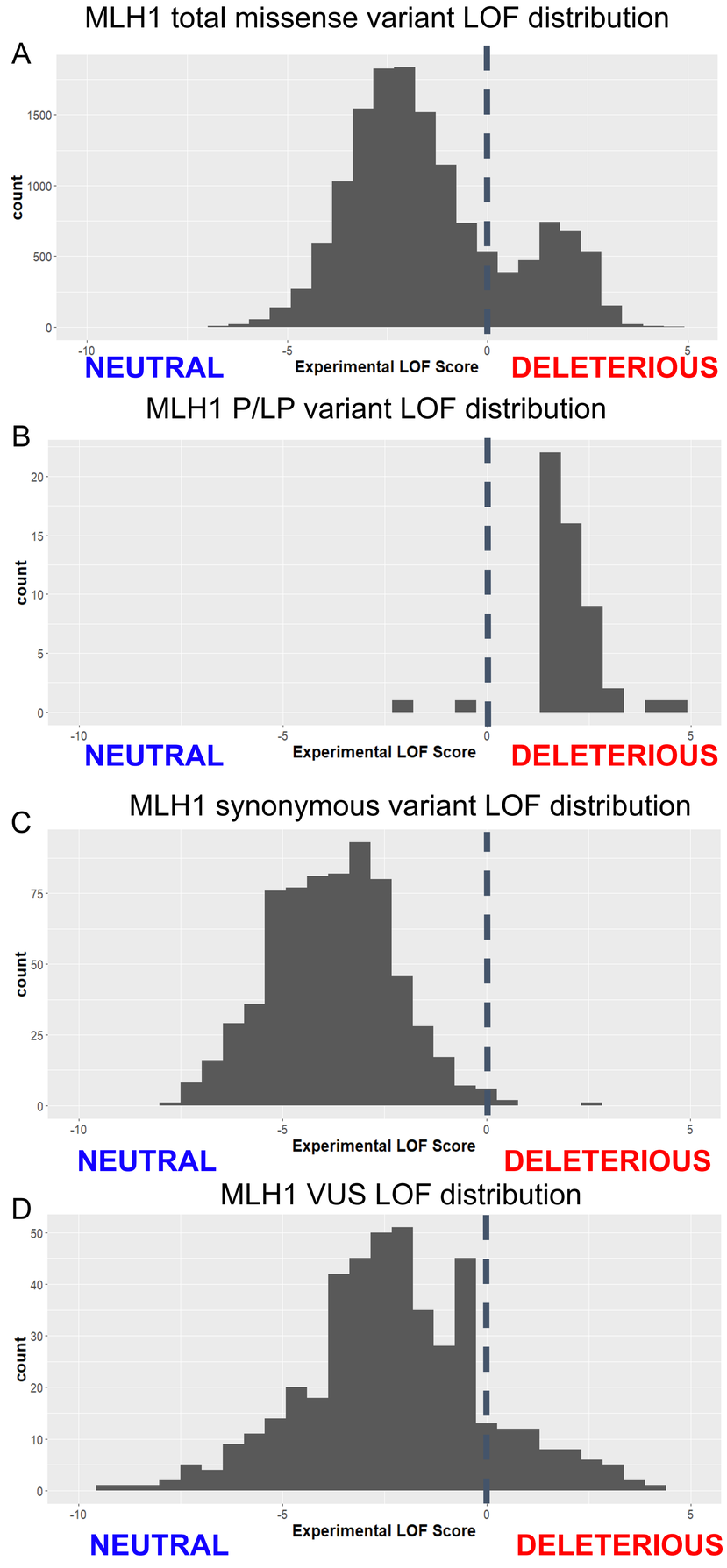


Figure 4: Histograms demonstrating LOF score distributions. Panel A shows the LOF score distribution of all missense *MLH1* variants, indicating that most mutations in this gene are predicted to be tolerated. Known pathogenic/likely pathogenic (P/LP) variants are shown in Panel B and enriched for deleterious LOF scores. Synonymous variants are almost exclusively neutral by this assay (Panel C). We then proceeded to overlay these scores on a population of variants of uncertain significance (VUS), the majority of which are predicted to have a neutral effect on MLH1 activity, reflecting the depletion of known pathogenic variants already identified in clinical testing (Panel D). The rate of VUS predicted to be pathogenic in this study (12.4%) is roughly consistent with reclassification rates in the clinical cancer genetics setting (~10%).

Results – Variant reclassification efforts

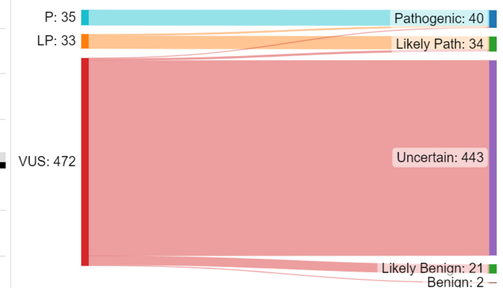


Figure 5: Alluvial diagram demonstrating variant reclassification. This cohort included 472 VUS missense variants in *MLH1,* of which a majority scored in the neutral range, consistent with incidentally discovered, benign rare variants unrelated to individual cancer history. Some of these had sufficient evidence for reclassification to benign/likely benign. Conversely, upgrades to pathogenic/likely pathogenic were pursued in select cases based a deleterious LOF score (>0.5) in conjunction with additional data. Figure made with SankeyMATIC.

Conclusions

- High-throughput assays for mismatch repair loss of function demonstrate excellent concordance with established variant classification
- DMS provide a scalable method for VUS resolution and, for deleterious LOF scores, serve as strong evidence criteria for pathogenicity (PS3)
- Deleterious LOF scores correlate well with other clinical evidence for pathogenicity

References:
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