

Title: Application of deep mutational scanning data for MLH1 variant interpretation

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Pathogenic germline variants in the DNA mismatch repair (MMR) pathway cause Lynch Syndrome (LS), a hereditary cancer predisposition syndrome affecting more than 1 in every 300 individuals worldwide. Effectiveness of LS genetic counseling is limited by the prevalence of variants of uncertain significance (VUS), which comprise the majority of missense variants identified by clinical genetic testing. We have established deep mutational scanning (DMS) as a scalable means for functional testing to support accurate variant interpretation in LS (Jia et al, AJHG, 2021; Scott et al, Genome Biol, 2022), which we now apply to the key gene MLH1. 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 Pathogenic (N=23) or Benign (N=27). All variants which exhibited normal 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. This cohort also included 590 VUS missense variants in MLH1, of which a majority (78%) scored in the normal range, consistent with incidentally discovered, benign rare variants unrelated to individual cancer history. By contrast, 12.4% of the clinical missense VUSs exhibited loss of function at the protein level and 5% were predicted to disrupt splicing: reclassification using the functional evidence for these 80 VUSs is ongoing. Saturation-scale functional testing via DMS can provide badly needed functional evidence to improve the actionability of genetic testing.