

Title: Evaluating Combinations of High-Throughput Evidence for Variant Classification in MSH2

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Background/Rationale: Updates to the 2015 v3 ACMG/AMP variant classification guidelines incorporate the point-based Bayesian framework published in 2020. However, the ability of evidence types, alone or in combination, to generate the strength of evidence assigned in the classification guidelines has not been validated. Here, we apply a maximum likelihood estimate model to combinations of high-throughput evidence for variants in MSH2 to empirically test assumptions in the classification guidelines.

Methods: We used clinical mutation screening data from Ambry Genetics from 59,138 individuals with colorectal and/or endometrial cancer and variation counts from 792,358 gnomAD v4.1.0 subjects to conduct our case-control analysis. Predictions from a nearly comprehensive MAVE and well-regarded computational tools, as well as exceptionally conserved ancestral residue (ECAR) status, were collected for snMS across MSH2. Variants were binned based on predictions of pathogenicity. Odds ratios and thence Bayesian points were calculated for each bin and compared to ACMG guideline expectations.

Results: Predictions of pathogenicity based on either MAVE results or ECAR status failed to meet ACMG expectations. Pathogenic predictions from the PRIORs database, the recommended computational tool, also did not meet ACMG expectations. A-GVGD and AlphaMissense were the only computational tools that generated sufficient evidence to meet guideline expectations. Nevertheless, variants that were predicted pathogenic by the MAVE and fell at an ECAR position exceeded ACMG expectations. Only A-GVGD was able to provide additional evidence in favor of pathogenicity when combined with MAVE results and ECAR status. Additionally, a large subset of variants (>75%) were predicted to be benign and had odds ratios close to 1, indicative of no increased risk of disease.

Discussion: Without rigorous calibration, application of Bayesian points to the evidence types used in variant classification is inappropriate. Each instance and combination of evidence needs to be carefully validated for the disease of interest prior to use. This validation should take place prior to the publication of new MAVES or computational tools. Furthermore, that greater than 75% of snMS have high-throughput evidence in favor of benignity, yet less than 10% of snMS are classified benign in ClinVar highlights the need to evaluate how clinical observational data weighs against high-throughput predictions.