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A comprehensive, clinically calibrated MAVE (multiplex assay of variant effect) for the human MutLα complex genes *MLH1* and *PMS2*.

DNA mismatch repair (MMR) is a key guardian of genome integrity, and its loss results in Lynch Syndrome (LS), an early-onset colon and endometrial cancer predisposition affecting >1:300 individuals worldwide. Four key mismatch repair factors (*MSH2*, *MSH6*, *MLH1* and *PMS2*) are widely screened in germline and tumor clinical testing, as reflected by the >13,000 missense variants listed in ClinVar for these genes. Of these, ~75% remain "variants of uncertain significance" (VUS), which hinder clinical management. To enable their rapid and accurate clinical interpretation, we developed MAVEs (multiplex assays of variant effect) to measure variant function across all MMR genes. Here, we present complete MAVEs for MLH1 and PMS2, which together form MutLα, the complex with endonuclease activity responsible for excising mismatched bases. Using a human-cell-based assay, we obtained functional measurements for >99% of the >30,000 missense variants across the two genes, of which we categorize 14.3% as exhibiting loss of function. Our MAVE scores achieve perfect (100%) concordance with high-confidence clinical variants in ClinVar (*PMS2*: n=439; *MLH1*: n=339). Under the ACMG/AMP OddsPath framework, these maps provide functional evidence at the "very strong" level. To investigate the association between the functional evidence and cancer risk, we intersected our MAVE maps with a large clinical genetic testing database containing ~950,000 individual, and found 13,334 carrying a rare missense variant in either gene. By leveraging ICD-10 codes for personal cancer history, we asked whether carriers of functionally abnormal missense variants had increased cancer risk compared to carriers of functionally normal variants. In *MLH1*, missense loss of function was associated with highly elevated risk for colorectal cancer (HR=5.37, p-val<1e-15) and endometrial cancers (HR=3.40, p-val<1e-11). For *PMS2*, we found lower but still significant risk (HR=2.20, p-val<1e-15; HR=2.75, p-val<1e-13, respectively), consistent with lower overall penetrance. As validation, we observed similar associations and effect sizes in NIH AllOfUs biobank. Finally, we leverage these complete MAVE maps to nominate key functional interaction residues by overlaying our MAVE scores onto an AlphaFold3-predicted complex. Our pathway-scale MAVE maps demonstrate how functional evidence can improve the accuracy and therefore actionability of genetic testing, and shed new insight into MMR functional mechanisms.