Germline mutations in the human DNA mismatch repair (MMR) genes MSH2 and MLH1 are associated with the inherited cancer disorder Lynch syndrome (LS). Up to 30% of MLH1 variants found are missense mutations. The functional consequences in regard to pathogenicity of many of these variants are unclear. Missense mutations affect protein structure or function, but may also cause aberrant splicing. In this study, *in silico* prediction tools (SIFT, PolyPhen-2 and ESEfinder 3.0), literature and online MMR mutation database review were used to evaluate the clinical significance of 26 MLH1 missense mutations identified in patients from Polish families, suspected of Lynch syndrome. Six MLH1 VUS were classified as pathogenic, nine MLH1 missense mutations are classified as neutral. 11 variants require additional research. The results show that *in silico* prediction tools can be utilized in an appropriate and efficient manner to determine the pathogenicity of MMR gene variations.