The R659Q and K618A *MLH1* variants (of uncertain significance and benign independently) are pathogenic when inherited in cis

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An Ohio family meeting Amsterdam criteria was seen by OSU Cancer Genetics several years ago (Figure 1). The proband with a history of metachronous endometrial and MSI-high colorectal cancers underwent genetic testing and was found to have the p.R659Q and p.K618A missense in the *MLH1* gene, which were initially both classified as uncertain variants. Segregation testing in the family identified two older unaffected relatives without the variants and 2 young unaffected family members with the variants proving that they were in cis. One of the mutations-positive unaffected individuals was subsequently diagnosed with two synchronous colorectal cancers on his first colonoscopy at age 26. The son of the proband presented to Cancer Genetics recently after being diagnosed with colorectal cancer at age 46. His tumor was MSI-high, absent MLH1 and PMS2 and did not have MLH1 promoter methylation. Paired tumor and normal testing originally indicated that he was negative but upon request it was confirmed that he had both the p.R659Q and p.K618A variants in MLH1 which had both been classified as likely benign/benign. The p.R659Q variant has now been submitted to ClinVar 8 times and is classified as likely benign x3, uncertain significance x4, and unclassified x1. The p.K618A variant has now been submitted to ClinVar 16 times and is classified as benign (x6), likely benign (x5), and not provided (x2). RNA studies did not reveal any aberrant splicing. Tumor sequencing did not provide additional information regarding pathogenicity. We have since identified at least three additional patients with this haplotype and clinical histories suggestive of Lynch syndrome: two from the same testing laboratory and one from the Colon Family Registry. Modified Bayesian analysis was performed including the segregation, MSI and IHC results from this family and a second family from the Colon Family Registry that also has both variants and the combined data was high enough odds to reach a pathogenic classification statistically [Prior P = 0.5; Tumor LR = 8.87 (2 x MMR deficient CRC without methylation) Segregation LR = 33.06 (FHCRC fam - 2.42 & OSU fam - 13.66) Posterior P = 0.997]. Upon review, p.R659Q was reclassified as a variant of uncertain significance until additional evidence is available to confirm that these two variants in cis are pathogenic. For example, additional functional studies and in-depth phenotypic comparisons of p.R659Q families with and without p.K618A could confirm pathogenicity

Conclusion: This case study highlights the challenges of identifying and interpreting complex alleles. While central repositories such as ClinVar have been an invaluable resource for variant interpretation,

there is limited ability to curate all co-occurring variants and track haplotypes that are potentially disease-causing. Patients with this combination of missense variants may be receiving negative results from commercial laboratories that have classified both as benign/likely benign as independent variants and at most may be receiving a single VUS result for the p.R659Q variant. If this combination of variants is proven to be pathogenic, it represents a new possibility that other combinations of otherwise benign missense variants may, in combination, be pathogenic.

Figure 1.