Multiple Mutations in Lynch syndrome: Case Report and Experience of One Clinical Laboratory

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Background: Lynch syndrome is typically caused by a single mutation in 1 of the mismatch repair (MMR) genes, *MLH1*, *MSH2*, *MSH6*, or *PMS2*, or by a gross deletion in *EPCAM*. Carrying 2 MMR mutations in *trans* is associated with a diagnosis of constitutional mismatch repair-deficiency syndrome (CMMR-D), which is characterized by colonic polyposis; hematologic, brain, and gastrointestinal malignancies; and neurofibromas, most often presenting in childhood. Limited data exist on the prevalence of individuals who have 2 MMR mutations not associated with CMMR-D.

Methods: To explore the prevalence and phenotype of patients with 2 MMR mutations, data was collected on 29,907 individuals sequenced for 2 or more MMR genes at Ambry Genetics as part of a multi-gene panel or targeted Lynch syndrome testing. Clinical histories were reviewed to assess the phenotypes of individuals carrying multiple MMR mutations.

Results: In this cohort of nearly 30,000 cases, probands from 16 families carried 2 mutations in the same MMR gene. In 1proband the mutations were confirmed to be in *cis* and this family had a classic Lynch syndrome presentation. Among 12 probands with homozygous mutations or mutations in the same gene confirmed in *trans,* all exhibited severe or CMMR-D consistent phenotypes. In the remaining 3 cases with 2 mutations in the same MMR gene, phase is unknown. We identified a single family with mutations in 2 different MMR genes. A 47-year-old woman presented with a history of five cancer primaries: adenocarcinoma *in situ* of the cervix at age 38, CLL at a 44, and bilateral invasive ductal carcinoma and endometrial adenocarcinoma at age 47. Immunohistochemical analysis of her endometrial adenocarcinoma revealed loss of *MSH6* protein expression. Her mother was diagnosed with bilateral breast cancer at age 57 and 72 and uterine cancer at age 61. Her maternal grandfather and second cousin were diagnosed with colon cancer at ages 66 and 47, respectively. Her paternal family history consisted of an aunt diagnosed with breast cancer at age 60, 1st cousin diagnosed with colon cancer at age 51, and grandfather diagnosed with prostate cancer in his 80s. The patient underwent next generation sequencing analysis with Ambry's CancerNext panel on a cultured fibroblast sample due to her history of CLL, and her mother also underwent CancerNext analysis. The proband was found to have an ***MSH6*** pathogenic mutation (c.3939_3957dup19), ***PMS2*** pathogenic mutation (c.736_741del6ins11), and ***MUTYH*** pathogenic mutation (p.G396D), along with an *APC* variant of unknown significance (p.R2166Q). The patient's mother also carries the *MSH6* and *PMS2* mutations and *APC* variant, but did not carry the *MUTYH* mutation.

Conclusions: In a cohort of nearly 30,000 cases tested for 2 or more MMR genes, we report 16 families with mutations in the same MMR gene, and a single family with mutations in 2 different MMR genes (*MSH6* and *PMS2*), where the affected individuals had phenotypes consistent with Lynch syndrome. While further studies are needed, this single report suggests that digenic mutations in MMR genes, specifically *MSH6* and *PMS2*, are not associated with a CMMR-D phenotype. These data also suggest that the presence of 2 MMR gene mutations is a rare event, occurring more often within the same gene than in 2 different genes.