Title: Discrepant tumor test results in individuals with Lynch syndrome identified by Next-Generation sequencing panels.

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Background: Tumor screening via microsatellite instability (MSI) and immunohistochemistry (IHC) is often utilized to rule out Lynch syndrome and guide molecular testing. While there are reports of individuals with Lynch syndrome and normal MSI/IHC, there are limited data on the prevalence of this. The purpose of this study was to assess the clinical histories and molecular characteristics of individuals with mismatch repair (MMR) and *EPCAM* gene mutations found on NextGen multi-gene panels.

Methods: Our sample consists of 112 patients with a MMR gene or *EPCAM* mutation identified by NGS multi-gene panel testing between March 2012 and March 2014. Results of tumor studies including MSI, IHC, MLH1 hypermethylation and BRAF V600E were assessed in individuals with pathogenic /likely pathogenic MMR gene mutations.

Results: Roughly 20% percent (22/112) of MMR mutation carriers in this cohort had prior tumor studies. The type of tumor included: colorectal (11), uterine (4), gastric (1), sebaceous adenoma (1) and not specified (5). Of the 22 tumor studies, 4 /22 individuals had MSI only, 2/22 had MSI and IHC while 16/22 individuals had IHC only and one had BRAF testing. Overall, 6/22 (27%) MMR mutation carriers who underwent screening with MSI and/or IHC had discrepant results. Two out of 6 (33%) cases were reported to be microsatellite stable (MSS), one with a *MSH6* mutation and one with a PMS2 mutation. Both also had normal IHC results. In addition 6/18 (33%) patients had discrepant IHC results, 1 with a *MLH1* mutation, 3 with *MSH6* mutations and 2 with *PMS2* mutations. We examined the effects of the mutation on the protein sequences and structures and in some cases can rationalize that some domains of the gene products may be stably expressed and folded. For example one missense mutation, p.N38H, in *MLH1* hydrogen bonds to ATP but may not lead to misfolding and protein instability and loss of antibody binding in IHC. Three out of the six (50%) patients with discrepant MSI/IHC met Amsterdam I, Amsterdam II or Bethesda criteria, while three individuals met NCCN guidelines for BRCA1/2 testing (one met both criteria) while one individual did not meet any criteria.

Conclusion: Our data demonstrate that although screening with MSI and IHC is an effective mechanism to identify individuals at high risk for MMR gene mutations, a significant portion (27%) of mutation carriers would be missed if we relied solely on tumor studies. Some mutations may lead to loss of function but not loss of all antibody binding in IHC, depending on the location of epitopes of the IHC antibodies and whether the mutation leads to degradation of the expressed protein. Given the variability of phenotypes in families with MMR mutations, and the clinical overlap with other syndromes such as *BRCA1/2*, NGS panel tests provide an effective way to test multiple genes at once and to identify individuals with mutations that would otherwise be missed by screening and family history alone. Additional studies are needed to help establish the prevalence of individuals with Lynch syndrome and normal tumor studies.