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Differentiating Lynch-like from Lynch Syndrome

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Lynch syndrome is a hereditary condition found in ~3% of all colorectal cancer patients and is defined by germline inactivation in one of the DNA mismatch repair (MMR) genes (*hMSH2*, *hMLH1*, *hMSH6*, *hPMS2*) (1,2). One allele is inactivated in every cell in a Lynch syndrome patient most commonly by pathogenic mutation or deletion of *hMSH2* or *hMLH1*, or less commonly by pathogenic mutation of *hMSH6* or *hPMS2*. In some Lynch syndrome patients, germline deletion of the 3' end of *EPCAM* (also known as *TACSTD1*) is found, and this deletion causes allele-specific methylation of *hMSH2* that is immediately upstream from *EPCAM* on chromosome 2, silencing the transcription of *hMSH2* (3). Patients with Lynch syndrome can develop synchronous and metachronous cancers at relatively young ages most commonly of the colon, but also of the endometrium and ovaries, the remainder of the gastrointestinal tract, the urinary tract, brain (glioblastomas), and specific skin cancers (Muir-Torre variant). With both the germline DNA MMR allele and the second somatic intra-cancer DNA MMR allele nonfunctional, DNA MMR enzyme activity is abated or lost within Lynch syndrome cancers. This allows multiple mutations to accumulate and advantages the tumor for growth and spread, and allows manifestation and detection of microsatellite instability (MSI) from within tumors that develop (4). Only a portion of Lynch syndrome patients and families might be initially identified by the clinically oriented Amsterdam criteria and/or Bethesda guidelines; most patients may be identified through MSI testing and DNA MMR protein immunohistochemistry (IHC) of the patient's tumor (1). However, germline testing for mutations within the DNA MMR genes is the ultimate the gold standard for characterizing Lynch syndrome families (1,2).

Lynch syndrome patients are not the only colon cancer patients for which their cancers manifest MSI. The largest group is sporadic colorectal cancer patients that possess acquired somatic hypermethylation of the promoter region of the DNA MMR gene *hMLH1* (5-8). This group accounts for ~15% of all colorectal cancer patients, and by the bi-allelic methylation of *hMLH1* promoter, *hMLH1* protein is not transcribed, and competency for DNA MMR functionality is lost (4). When performing MSI testing and DNA MMR protein

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IHC on these cancers, it may be difficult to tell if the cancer is from a sporadic patient or potentially a Lynch syndrome patient. Some differentiating features between a sporadic hypermethylation *hMLH1* cancer and a germline *hMLH1* Lynch syndrome cancer, both of which manifest MSI, are: (a) the presence of *BRAF* mutations in sporadic cancers, (b) the older age at diagnosis in sporadic cancer patients, (c) the lack of significant family history suggesting of Lynch syndrome in sporadic patients, and (d) the presence of methylation on the *hMLH1* promoter in sporadic patients (1-3). Another group whose cancers manifest MSI are the recently described Lynch-like syndrome patients (9-11). This group may account for as much as 70% of suspected Lynch syndrome patients (9). Unlike sporadic MSI cancer patients, Lynch-like patients are nearly impossible to differentiate from Lynch patients: they manifest MSI within their cancers, and the cancers show abnormal DNA MMR protein IHC – not only for *hMLH1* as with sporadic MSI cancers, but also for the other DNA MMR proteins like *hMSH2*, *hMSH6* and *hPMS2* as with true Lynch syndrome cancers (9). Additionally, Lynch-like syndrome patients have a mean age of onset similar to Lynch syndrome patients (53.7 years of age vs. 48.5 years of age) (9). The only differentiating features between these two syndromes that have been described to date are the lower standardized incidence ratio (SIR) in Lynch-like syndrome compared to Lynch syndrome for colorectal cancer (2.12 vs. 6.04) and non-colorectal cancer Lynch syndrome-associated cancers (1.69 vs. 2.81), and the absence of an identifiable DNA MMR gene germline mutation in Lynch-like syndrome (9). The mechanism for the generation of MSI within Lynch-like cancers is Unknown. In this issue of GASTROENTEROLOGY, Mesenkamp, *et al.* (12) make significant headway in identifying the mysterious cause for inactivating DNA MMR function and subsequent MSI generation within Lynch-like syndrome cancers.

Cancers from Lynch-like syndrome patients show MSI, have no DNA MMR gene mutation detected in their germline, and show no hypermethylation of *hMLH1* as a cause for the MSI (9, 11). There are likely three potential reasons for cancers from Lynch-like patients to show MSI but no DNA MMR germline mutation: (a) there are unknown gene mutations other than the DNA MMR genes in the germline that can drive MSI, (b) there are germline mutations in the DNA MMR genes that are not identified by detection methods used, and/or (c) there is a genetic process within the tumors other than germline mutation coupled with the second allele inactivation or bi-allelic hypermethylation of *hMLH1* that causes Lynch-like cancers to manifest MSI. It is possible that all of these possibilities could be at play for Lynch-like syndrome as they are not exclusive mechanisms from each other, and given the intermediate colorectal cancer and other non-colorectal cancer standardized incidence ratios squarely between the ratios for Lynch syndrome and sporadic colorectal cancer, Lynch-like syndrome may be a heterogeneous condition between these two extremes. In evaluating the three possibilities, the first regarding an unknown germline gene driving MSI is the most remote. The DNA MMR apparatus is well studied, and most associated components are known, with no other reports of germline mutations outside of these genes other than *EPCAM* as described above (3,4). The second possibility of missing germline mutations within the DNA MMR genes is plausible. Current approaches sequence the exon components of these genes, and utilizes technology that detect some deletions that are commonly observed for some families for *hMSH2* and *hMLH1*. *EPCAM* mutations were discovered as a driver to methylate the neighboring *hMSH2* gene(3). There could be similar

processes that are yet to be discovered that affect other DNA MMR genes. Promoter and intron portions of the DNA MMR genes are not routinely assayed for mutation, unusual inversions might occur (13), and a full understanding of genetic variants within these genes is not complete. Thus, missing mutations in the DNA MMR genes, which would make Lynch-like patients Lynch patients, is a real possibility.

Mesenkamp, *et al.* now address the third possibility and show this to be the cause in at least half of Lynch-like syndrome cancers. In their study, the authors determined to what extent somatic mutations within *hMSH2* and *hMLH1* could explain the presence of MSI among a cohort containing 232 MSI-positive colorectal and endometrial cancers. In 181 cancers, the underlying cause for DNA MMR deficiency was identified (*hMLH1* promoter hypermethylation or *hMLH1*, *hMSH2*, *hMSH6*, or *hPMS2* germline mutation), leaving 51 cancers with MSI unexplained. After eliminating cancers in which the IHC pattern indicated *hMSH6* or *hPMS2* loss, or issues with DNA quality or patient consent for somatic sequencing, 25 cancers were evaluated for somatic mutation of *hMLH1* and *hMSH2*. Among this remaining group, 8 *hMLH1*-deficient cancers and 5 *hMSH2*-deficient cancers as determined by IHC demonstrated two convincing pathogenic somatic hits (for a total of 13/25, or 52%). The most common two-hit combination was mutation coupled with loss of heterozygosity (LOH), followed by two somatic mutations. The percentage for two somatic hits in these Lynch-like tumors may be an underestimate, as combinations of a pathogenic mutation or LOH with variants of uncertain significance or uninformative LOH analysis were present but are uncertain whether these are the cause for the MSI phenotype. One other group identified that bi-allelic mutation of DNA MMR genes was possible (10), and the work of Mesenkamp, *et al.* shows that with careful analysis that this occurs in half of Lynch-like cancers.

What does this mean for Lynch-like patients? Are they the equivalent of sporadic colorectal cancer patients? Should they undergo surveillance for early cancer detection? The data from Mesenkamp, *et al.* does not eliminate the possibility of a germline cause for Lynch-like syndrome. Their data simply show that the cause of MSI in their tumors is from two somatic events that affect DNA MMR gene expression. Their data, along with previously published data (9) suggests that Lynch-like patients present at younger ages than sporadic MSI colorectal cancer patients, and in the Mesenkamp, *et al.* study, the mean age for diagnosis of patients with two somatic hits was not different than Lynch syndrome patients with *hMLH1* or *hMSH2* germline mutations. This means that even for the group that has 2 identified somatic DNA MMR gene events, plus the group of Lynch-like patients that do not show two events, that Lynch syndrome is still a possible diagnosis for these patients, and it remains prudent to continue to perform surveillance for cancer formation in these patients. While it is important to identify Lynch syndrome from sporadic cases of colorectal cancer as patients with Lynch syndrome have recurring bouts of cancer development, the separation between Lynch-like and Lynch syndrome, to date, is not as clear cut. As shown in the **table**, there are a number of recognized mechanisms to inactivate DNA MMR function (4-8,10,12-18), several of which do not directly affect the sequence of the DNA MMR genes. These and other mechanisms could be operative in the germline of Lynch-like patients. It is also possible that Lynch-like patients may represent a heterogeneous group between sporadic

MSI patients and true Lynch patients. Lynch-like syndrome is a new and evolving condition, and studying this group will ultimately help appropriately categorize their family risk and surveillance approaches. For now, half or possibly more of Lynch-like cancers have their MSI occurrence explained.

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Table

DNA mismatch repair (MMR) defects within heritable and sporadic colorectal cancers.

	MMR Germline Mutation	MMR Somatic Mutation/LOH	MMR Epigenetic Inactivation	Other
Lynch syndrome	1 st allele (<i>hMLH1</i> , <i>hMSH2</i> , <i>hMSH6</i> , <i>hPMS2</i> , <i>EPCAM</i>); 2 nd allele (<i>hMLH1</i> , <i>hMSH2</i> , <i>hMSH6</i> , <i>hPMS2</i>) in Constitutional MMR Deficiency Syndrome	2 nd allele (<i>hMLH1</i> , <i>hMSH2</i> , <i>hMSH6</i> , <i>hPMS2</i>)	Allele-specific <i>hMSH2</i> methylation in tissues expressing <i>EPCAM</i> as a consequence of neighboring germline 3' end <i>EPCAM</i> deletion Constitutional epimutation of <i>hMLH1</i>	None reported
Lynch-like syndrome	None	1 st and 2 nd alleles (<i>hMLH1</i> , <i>hMSH2</i> , <i>hMSH6</i> , <i>hPMS2</i>)	None reported	None reported
Sporadic MSI	None	Secondary <i>hMSH3</i> and <i>hMSH6</i> mutations as a consequence of <i>hMLH1</i> promoter methylation	Bi-allelic promoter methylation for <i>hMLH1</i>	<i>hMSH2</i> protein deficiency due to deletions of <i>FRAP1</i> (<i>MTOR</i>), <i>HERC1</i> , <i>PRKCZ</i> or <i>PIK3C2B</i> that regulate <i>hMSH2</i> protein degradation H3K36 trimethyltransferase SETD2 deficiency prevents formation of H3K36me3 histone sites for hMutS α to bind to chromatin, preventing hMutS α function
EMAST	None	None reported	None reported	Nuclear-to-cytosol shift for <i>hMSH3</i> protein

MSI = microsatellite instability; EMAST = elevated microsatellite alterations at selected tetranucleotide repeats; LOH = loss of heterozygosity; hMutS α is a heterodimer between *hMSH2* and *hMSH6* proteins.