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Risk assessment and genetic counseling for Lynch syndrome - Practice Resource of the National Society of Genetic Counselors and the Collaborative Group of the Americas on Inherited Gastrointestinal Cancer

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Introduction

Lynch syndrome (LS) is an autosomal dominant hereditary cancer condition caused by heterozygous germline pathogenic/likely pathogenic variants in the mismatch repair (MMR) genes: *MLH1*, *MSH2*, *MSH6*, and *PMS2*, as well as 3' terminal deletions of *EPCAM*. LS primarily predisposes to colorectal cancer (CRC) and endometrial cancer (EC), accounting for ~3% of each cancer (Hampel et al., 2005; Hampel et al., 2006). It is estimated that germline pathogenic/likely pathogenic variants in the MMR genes are identified in 1 in 300 individuals (Win et al., 2017). The hallmark feature of LS-related tumors is deficient MMR (dMMR) activity, which can be assessed through MMR immunohistochemistry (IHC) and/or microsatellite instability (MSI).

Diagnosis of LS has rapidly evolved due to universal tumor screening recommendations and the advent of next-generation sequencing (NGS). The setting in which individuals and families with LS are now being identified has expanded outside of the traditional genetic counseling and testing model. Individuals being assessed for or

identified with LS should be offered evaluation by a genetics professional with expertise in cancer risk assessment. This practice resource will provide updates on best practices when evaluating these individuals and families. Management for LS has been reviewed elsewhere and will not be addressed in this document (NCCN, 2021; Giardiello et al., 2014; Stjepanovic et al., 2019).

Background

Nomenclature

Historically, the terms LS and hereditary non-polyposis colorectal cancer (HNPCC) had been used interchangeably and were based on family history criteria, such as Amsterdam I and II criteria, regardless of tumor or germline variant status (Boland, 2005; Jass, 2006; Vasen et al., 1991, Vasen et al., 1999). LS is the currently accepted designation for individuals with a confirmed heterozygous germline pathogenic/likely pathogenic variant in one of the MMR genes or a 3' terminal deletion of *EPCAM,* regardless of family history (Box 1) (Weissman et al., 2011). The terms Muir-Torre syndrome and Turcot syndrome, previously used to define variants of LS that include sebaceous gland neoplasms and/or keratoacanthomas and glioblastoma multiforme, respectively, are outdated and are understood to fall under the definition of LS (Box 1) (Weissman et al., 2011). Other family history-based terms such as "familial colorectal cancer type X", families that meet Amsterdam I criteria but with proficient MMR (pMMR) tumors, are a separate defined group and are not LS (Lindor et al., 2005).

The terms "Lynch-like syndrome" or "mutation-negative LS" have been used to define tumors with dMMR but no germline pathogenic/likely pathogenic variant in a MMR gene or *EPCAM* (Rodriguez-Soler et al., 2013; You & Vilar, 2013). This nomenclature is misleading and any use of the term "Lynch syndrome" for this group should be avoided (Box 1). This group is most frequently non-hereditary in nature, with the majority of these dMMR tumors attributable to biallelic somatic inactivation of the MMR genes, and therefore unrelated to inherited LS (Haraldsdottir et al., 2014; Mensenkamp et al., 2014).

Constitutional mismatch repair deficiency (CMMRD) is a rare syndrome due to biallelic inheritance of germline pathogenic/likely pathogenic variants in the same MMR

gene resulting in complete loss of MMR function (Box 1) (Bakry et al., 2014). Individuals with CMMRD typically present with early onset and often childhood cancers, most frequently of the brain, gastrointestinal tract, and hematologic system, as well as a phenotype that mimics neurofibromatosis type 1 (Tabori et al., 2017; Wimmer et al., 2014).

Clinical Features

LS primarily causes an increased risk for CRC and EC, but also predisposes to a range of other cancers such as ovarian, stomach, small bowel, urothelial, pancreatic, biliary, brain and sebaceous cancers. The lifetime cancer risk estimates (penetrance) have changed over time. Initial estimates were based on family history and derived from families with high cancer incidences that met strict family history criteria which led to ascertainment bias. These families may not have had confirmed germline MMR pathogenic/likely pathogenic variants. Early penetrance estimates may be based on data from *MLH1* or *MSH2* only and often grouped all MMR genes together. Utilization of universal tumor screening and multi-gene panel testing has led to the identification of individuals with LS who may have a less significant personal or family history of cancer than reported in earlier LS cohorts. This has decreased penetrance estimates and redefined what an “average” LS family looks like (Box 2).

The penetrance for LS-related tumors varies based on gene and sex (Table 1 and Box 2) (Moller et al., 2017; Bonadona et al., 2011; Choi et al., 2009, van der Post et al., 2010; Baglietto et al., 2010; Engel et al., 2012; NCCN, 2021). The risk for CRC is highest for *MLH1* and *MSH2* (Ramsoekh et al., 2009). The penetrance is estimated to be in the 60% range for both genes (Moller et al., 2017; Bonadona et al., 2011; Choi et al., 2009, van der Post et al., 2010; Baglietto et al., 2010). The risk for CRC is lower with *MSH6* and *PMS2* and is estimated to range from 12-31% (Bonadona et al., 2011; Baglietto et al., 2010; ten Broeke et al., 2018; Senter et al., 2008; Dominguez-Valentin et al., 2020).

The gynecologic cancer risk for *MSH6* is estimated to be higher than the risk of CRC (Dominguez-Valentin et al., 2020). EC occurs in 13-57% of women with LS (Table 1) (Moller et al., 2017; Bonadona et al., 2011; Baglietto et al., 2010). The second most common extra-colonic cancer in women with LS is ovarian cancer with a range of 3-

20%. Recent studies indicate a variation in risk that is gene-specific (Table 1) (Moller et al., 2017; Bonadona et al., 2011; Baglietto et al., 2010).

The LS tumor spectrum also includes other non-gynecologic extra-colonic cancers (Table 1). The overall incidence of extra-colonic cancers appears to be highest in *MSH2*, particularly for urothelial cancer (Therkildsen et al., 2017; Vasen et al., 2001). Other less common LS-associated cancers include sebaceous carcinoma, pancreatic, and hepatobiliary cancer (Dominguez-Valentin et al., 2020).

Increased risk for breast and prostate cancer in LS have been suggested but no definitive conclusions have been made (Box 2) (Roberts et al., 2018; Stoll et al., 2020; Ryan et al., 2014). Penetrance estimates from recent studies have not demonstrated an increased risk for breast cancer among women with pathogenic/likely pathogenic variants in MMR genes (Dominguez-Valentin et al., 2020; Stoll et al., 2020). Studies of the association of prostate cancer in LS indicate a possible increase in risk, with some studies suggesting up to a 30% lifetime risk, although this may be confined to *MSH2* alone. (Ryan et al., 2014; Haraldsdottir et al., 2014; Dominguez-Valentin et al., 2020).

Comparative penetrance estimates from clinic-based versus population-based families have shown that penetrance of pathogenic/likely pathogenic variants is variable and likely influenced in part by the impact of the particular pathogenic/likely pathogenic variants on gene expression, protein expression, and MMR function, shared familial/genetic factors, environmental risks, and other factors (Dominguez-Valentin et al., 2021; Win et al., 2021).

Diagnostics

Clinical Testing Criteria

The diagnostic criteria for identifying LS have continued to evolve since they were first developed. The International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer developed the Amsterdam Criteria for HNPCC in 1991 in order to standardize patient recruitment for research studies (Vasen et al., 1991). Amsterdam I was based on personal and family history of CRC (≥3 individuals affected, one a first-degree relative of the other two, in ≥2 successive generations, ≥1 case diagnosed under age 50; with familial adenomatous polyposis excluded). Criteria was expanded in 1999 (Amsterdam II) to include additional LS-associated tumors: endometrial, small

bowel, and urothelial (Vasen et al., 1999). The Amsterdam criteria have high specificity, but low sensitivity, and it became evident that many individuals with germline pathogenic/likely pathogenic variants in MMR genes have family history that does not meet Amsterdam I or II criteria (Hampel et al., 2008). LS-associated CRCs exhibit molecular phenotypes of dMMR and the Bethesda guidelines were established, and revised in 2004, to set criteria for which CRCs should undergo MSI analysis (Umar et al., 2004). Although the Bethesda guidelines increased clinical use of tumor testing, strategies which employed universal screening of unselected CRCs ultimately emerged as the most effective/cost-effective approach for identifying individuals with LS (EGAPP, 2009). Universal LS tumor screening was endorsed in the United States in 2009 by the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) working group. Universal tumor screening has expanded beyond just testing CRC and is now endorsed by multiple professional groups around the world (Box 3) (NCCN, 2021, Stjepanovic et al., 2019; Yamazaki et al., 2019). Expanding tumor analysis to the spectrum of non-CRC LS-associated cancers provides additional opportunities to identify individuals whose tumors develop in the setting of LS (Latham et al., 2019).

Clinical guidelines for identifying individuals at risk for LS endorse approaches that combine personal and family history assessment with tumor data when available (Box 3). Red flags include CRC or EC diagnoses at young age (<50), multiple primary cancers and/or dMMR tumors, at any age, and/or multiple relatives affected with LS-associated tumors. Several risk prediction models have been developed such as MMRPro (Chen et al., 2006), MMRPredict (Barnetson et al., 2006), Leiden (Wijnen et al., 1998) and PREMM1,2,6 updated to PREMM5 (Kastrinos et al., 2017), which incorporate personal and family history to quantify the probability that an individual carries a germline MMR pathogenic/likely pathogenic variant. At a threshold of ≥5%, these models have comparable test characteristics (Win et al., 2013; Khan et al., 2011) with variable ease of use and accessibility. The suggested testing threshold for the newest model, PREMM5, is ≥2.5% based on optimal sensitivity, though there are concerns about how this threshold applies in a general population of unaffected individuals (NCCN, 2021), highlighting the need for more data. The PREMM model has been successfully implemented in a number of clinical settings, facilitating the

diagnosis of LS among both cancer-affected as well as cancer-unaffected individuals (Luba et al., 2018; Guivatchian et al., 2017).

Microsatellite Instability

MSI is a characteristic found in 12-13% of all CRCs (Salovaara et al., 2000; Hampel et al., 2008) and 17-23% of all ECs (Modica et al., 2007). The three main causes of dMMR that can lead to MSI are: 1) acquired *MLH1* promoter hypermethylation, 2) LS due to a germline MMR pathogenic/likely pathogenic variant followed by acquired inactivation of the second MMR allele, and 3) acquired biallelic inactivation of the same MMR gene.

There are a variety of combinations of microsatellites which can be evaluated in tumors to detect MSI. MSI has traditionally been evaluated using PCR testing for a defined set of microsatellites such as those established by the Bethesda guidelines (Umar et al., 2004). Currently the most widely utilized combination is a five-microsatellite panel consisting of mononucleotide repeat markers: BAT-25, BAT-26, NR-21, NR-24, and MONO-27. A tumor is MSI-high (MSI-H) if ≥40% of microsatellites are unstable, microsatellite-stable (MSS) if no repeats are unstable, and MSI-low (MSI-L) if a tumor has instability, but at <40% of the repeats. The sensitivity and specificity of MSI by PCR for identifying CRC patients with LS is 77-91% and 90.2% (Palomaki et al., 2009). The sensitivity and specificity of MSI by PCR for identifying EC patients with LS is 77-100% and 38-81% (Lu et al., 2007; Mercado et al., 2012; Berends et al., 2003).

MSI can also be detected by NGS on tumor DNA. Several different methods have been published (Kautto et al., 2017; Middha et al., 2017; Niu et al., 2014; Salipante et al., 2014; Stadler, et al., 2016) and all rely on far more than five microsatellites. Sensitivity (76.1-97.2%) and specificity (98.7-99.7%) is high for all of the published NGS methods when compared to MSI by PCR (Kautto et al., 2017).

Immunohistochemistry

IHC of the MMR proteins on tumor and paired normal samples has been shown to be an acceptable substitute for MSI as concordance rates between MSI and IHC are 94% in both CRC and EC (Palomaki et al., 2009). IHC provides additional information over MSI as it allows MMR gene-specific DNA analysis depending on the staining pattern, but this has become less important now that germline genetic testing routinely

involves a NGS panel of multiple cancer susceptibility genes including all the MMR genes. Generally, either MSI or IHC is selected for universal tumor screening programs to decrease the cost. However, for high-risk individuals, performing both MSI and IHC tests may minimize the chance of missing a diagnosis of LS (Box 4). Absence of one or more of the MMR proteins occurs in 15% of unselected CRCs (Hampel, 2010) and 21% of unselected EC (Backes et al., 2009). The majority of cancers with abnormal IHC will exhibit a staining pattern of MLH1 and PMS2 deficiency. This pattern most likely indicates sporadic *MLH1* promoter hypermethylation, although this pattern is also seen when there is a germline pathogenic/likely pathogenic variant in *MLH1*. *MLH1* promoter hypermethylation has been found in tumors with PMS2 deficiency only, particularly in ECs (Kato et al., 2016). Concurrent MSH2 and MSH6 deficiency indicates a possible germline *MSH2* pathogenic/likely pathogenic variant or a 3' terminal deletion of *EPCAM* (Lightenberg et al., 2009). Isolated loss of MSH6 or PMS2 indicates a possible germline pathogenic/likely pathogenic variant in either respective gene. All these abnormal IHC findings can also be caused by biallelic somatic inactivating variants in the MMR genes.

Studies have shown that the sensitivity and specificity of IHC to identify LS among CRC patients is 83-93% and 88.8% (Palomaki et al., 2009; Hampel et al., 2005; Hampel et al., 2008). The sensitivity and specificity of IHC to identify LS among EC patients ranges from 86-100% and 48-67%, respectively (Lu et al., 2007; Mercado et al., 2012; Berends et al., 2003). IHC when used in conjunction with MSI may accurately identify close to 100% of LS-related CRCs (Lindor et al., 2002; Hampel et al., 2005). Concordance rates between MSI and IHC for ECs are the same as for CRCs, suggesting that IHC is a reliable test for evaluating individuals with EC for LS (Modica et al., 2007).

The IHC staining pattern in tumors from individuals with CMMRD differs from the typical pattern seen in LS-related cancers, as both the tumor and normal tissue can exhibit absent protein. Often this pattern may be difficult to interpret as there is no "normal" tissue to use as an internal positive control. Review by an experienced pathologist may help determine the true CMMRD cases from those in which the IHC staining has failed.

MSI and IHC in other tumors

MSI can be found in any tumor type, but has been used most commonly to screen CRC and EC. MSI is being included routinely in tumor sequencing tests since the FDA approved anti-PD-1/L1 therapy for tumors that exhibit a MSI-H phenotype. A study that included 15,045 patients with over 50 cancer types with paired tumor and germline sequencing found that 2.2% were MSI-H, 4.6% were MSI-Intermediate, and 93.2% were MSS (Latham et al., 2019). Although CRC and EC comprised only 9% of all tumors, these LS-related tumors represented 62% of the MSI-H cohort. The overall prevalence of LS was 16.3% among patients with MSI-H tumors, 1.9% among patients with MSI-intermediate tumors, and 0.2% among patients with MSS tumors (Latham et al., 2019). As a result, any patient with an MSI-H tumor should be referred to a cancer genetics clinic for genetic counseling and consideration of genetic testing regardless of whether or not the tumor is part of the LS tumor spectrum.

BRAF and *MLH1* Promoter Hypermethylation Testing

Somatic inactivation of the MMR genes can be a cause of MSI in sporadic cancers. Both somatic *MLH1* promoter hypermethylation, an epigenetic change, and somatic mutations of the *BRAF* gene have been described in sporadic CRCs exhibiting MSI and/or loss of MLH1 expression (Box 4) (Loughrey et al., 2007). These somatic events are rarely seen in LS-related CRC and therefore may be useful in determining whether a MSI-H CRC is more likely to be sporadic.

Somatic mutations of *BRAF* have been described in CRC, particularly in those that are MSI-H, proximal, and diagnosed at a later age of onset; this is also true of *MLH1* promoter hypermethylation. The *BRAF* V600E mutation has been shown to correlate with somatic *MLH1* promoter hypermethylation and not germline pathogenic/likely pathogenic variants in MMR genes (Wang et al., 2003). The positive predictive value of *BRAF* V600E indicating somatic *MLH1* promoter hypermethylation is estimated to be 99%, whereas the negative predictive value is only 41% (Adar et al., 2017). MSI –H CRCs without the *BRAF* V600E mutation may still have either *MLH1* promoter hypermethylation, have a germline *MLH1* pathogenic/likely pathogenic variant, or have acquired biallelic inactivation of the *MLH1* gene.

Another approach to assessing *MLH1* promoter hypermethylation is the use of BRAF IHC. This option can be especially helpful for small tumors without enough

material for DNA extraction in order to perform molecular testing. The presence of the BRAF protein indicates that an activating *BRAF* mutation is present (Toon et al., 2013). When compared to molecular *BRAF* testing, BRAF IHC has a sensitivity of 35-89% and specificity of 51-100% (Toon et al., 2013; Roth et al., 2015). At this time, many recommend that BRAF IHC should only be used when molecular *BRAF* testing is not possible (Bellizzi, 2015; Kwon et al., 2018; Reagh et al., 2018).

More than 70% of ECs that are MSI-H may be due to *MLH1* promoter hypermethylation (Simpkins et al., 1999). However, unlike CRC, several studies indicate that *BRAF* mutations are rare in MSI-H EC (Weissman et al., 2011). Therefore, methylation studies, not *BRAF* analysis, is the only effective method with which to determine if an MSI-H EC is due to *MLH1* promoter hypermethylation (Box 4).

Several cases of constitutional *MLH1* epimutations have been described, in which the promoter of one of the *MLH1* alleles is hypermethylated in the germline, resulting in transcriptional silencing of this allele in non-neoplastic tissue (Hitchins & Ward, 2009). Constitutional *MLH1* epimutations are typically found in CRCs that are MLH1/PMS2 deficient via MMR IHC, with *MLH1* promoter hypermethylation in the adjacent normal tissue as well as the tumor, with no *BRAF* V600E mutation, and no *MLH1* germline pathogenic/likely pathogenic variant (Box 4). Individuals with constitutional *MLH1* epimutations often do not have a significant family history of cancer as they frequently arise de novo, but heritable epimutations in some families with a significant family history of cancer have been reported (Hitchins et al., 2007; Hitchins et al., 2011). Detection of constitutional *MLH1* epimutation requires methylation testing to be undertaken in blood, saliva, or other non-neoplastic tissue sample.

Biallelic Somatic MMR Inactivation

It has been shown that 52-69% of dMMR CRCs and ECs without *MLH1* promoter hypermethylation and germline negative genetic testing are due to presumed biallelic somatic inactivation in the MMR genes (Geurts-Giele et al., 2014; Haraldsdottir et al., 2014; Mensenkamp et al., 2014). However, MSI and IHC alone cannot predict which individuals have LS versus those which have biallelic somatic MMR inactivation. For patients with dMMR CRC, factors predictive of LS include having more than one LS malignancy or a positive family history of LS-related tumors (Pearlman et al., 2019). The

only factor predictive of having biallelic somatic MMR inactivation was absence of MLH1 and PMS2 on IHC (Pearlman et al., 2019). Age was not predictive of LS versus biallelic somatic MMR inactivation. Table 2, adapted from Perlman 2019, provides the likelihood that a CRC patient with a dMMR tumor will have LS, biallelic somatic MMR inactivation, remain unexplained after tumor testing, or have false abnormal IHC based on their IHC findings.

Patients with dMMR EC, without MLH1 methylation, are more likely to have biallelic somatic MMR inactivation than LS (Hampel et al., 2021). The only IHC finding that is more likely to result in a LS diagnosis than biallelic somatic MMR inactivation in EC is absence of MSH6 on IHC.

Paired tumor and germline MMR gene sequencing can be useful in determining whether the cause of a dMMR tumor is LS or biallelic somatic MMR inactivation (Box 4). This can be helpful to determine recommendations for cancer surveillance or risk-reducing surgery; whether the family should follow LS guidelines or should be given management recommendations based solely on family history. It can be very reassuring to prove that an individual has biallelic somatic MMR inactivation and not LS (Haraldsdottir et al., 2014; Mensenkamp et al., 2014). It is important to note that when doing paired tumor and germline testing, the germline test should include the LS genes as well as additional CRC genes as it is known that individuals with biallelic *MUTYH* pathogenic/likely pathogenic variants that cause *MUTYH*-associated polyposis (MAP), as well as other germline pathogenic/likely pathogenic variants in DNA repair genes, can develop somatic MMR inactivation leading to dMMR (Morak et al., 2014).

Tumor profiling

The use of NGS-based tumor genomic profiling to assess an individual's cancer for targets of precision therapies represents an increasingly common clinical scenario through which germline MMR pathogenic/likely pathogenic variants may be identified. Recognition of clinically actionable targets, such as MSI, across a wide diversity of tumors and FDA approval of the use of targeted agents such as anti-PD-1/L1 monoclonal antibodies, have increased the relevance of conducting tumor genomic profiling in nearly every adult cancer patient, especially those with incurable disease. As such, individuals with a diversity of tumor types, including those not classically related to

LS, will undergo somatic sequencing of the MMR genes by way of tumor genomic profiling. While the MSI phenotype is most common among cancers within the classic LS spectrum, tumors exhibiting MSI may be found in nearly every cancer histology (Chalmers et al., 2017; Salem et al., 2020), and a portion of these will have germline MMR pathogenic/likely pathogenic variants (Box 5) (Latham et al., 2019). The Latham et al. study discussed previously demonstrated that tumors that are not classically associated with LS can be MSI-H and have germline pathogenic/likely pathogenic variants (2019) and should be referred to a cancer genetics clinic for genetic counseling and consideration of genetic testing regardless of whether the tumor is part of the LS tumor spectrum (Box 5). Up front tumor genomic profiling may, in the near future, supersede the role of universal MMR screening of CRC by IHC or MSI due to its high sensitivity, specificity, and efficiency in detecting germline pathogenic/likely pathogenic variants in the MMR genes and other clinically relevant variants in CRCs (Hampel et al., 2018).

The identification of somatic MMR variants by tumor genomic profiling presents challenges in determining whether an individual in fact has LS. One challenge relates to whether tumor genomic profiling is conducted with a matched normal blood sample or not. Without this, a laboratory will be unable to determine with certainty whether a particular somatic MMR variant is present in the germline or not, necessitating follow-up germline testing to diagnose LS. Laboratories that use a matched normal blood sample for germline variant filtering may also lack CLIA certification to report germline findings, leading to tumor genomic profiling reports that lack information regarding the somatic versus germline status of a MMR variant. Data from several studies demonstrate that the majority of MMR pathogenic/likely pathogenic variants identified by tumor genomic profiling are somatic only (Meric-Bernstam et al., 2016; Schrader et al., 2016), and thus a matched blood sample may be extremely helpful in determining which individuals require genetic counseling and germline testing. Providers must also be cautioned that pathogenic/likely pathogenic variants in the MMR genes, while more likely to be found in a tumor type associated with LS, may be identified in any tumor type on tumor genomic profiling (Box 5). Germline status cannot be determined based on whether the tumor being analyzed is part of the LS tumor spectrum or not. Some factors may help inform

on the likelihood of whether a variant is somatic or germline such as age of diagnosis, family history of LS-related tumors, MSI, MMR IHC, *MLH1* methylation status, and variant allele frequency around 50%; range could be 30-70% depending on reporting institution. However, none of these factors are definitive on determination of germline status and we would recommend that referral for germline confirmation of a MMR pathogenic/likely pathogenic variant be offered (Box 5).

Tumor tests such as MSI, MMR IHC, and tumor genomic profiling alone cannot definitively determine an inherited cause to a tumor and this is why some of these tests may not require specific patient informed consent. Tumor genomic profiling often includes a matched normal sample, which can provide definitive evidence for a germline pathogenic/likely pathogenic variant. Due to this, the standard of care proposed by the American Society of Clinical Oncology recommends that before tumor genomic profiling is conducted, individuals should be informed of the potential to identify germline pathogenic/likely pathogenic variants that may diagnose LS or other hereditary cancer syndromes, and should be offered the option to opt out of being given this information (Box 5) (Robson et al., 2015).

Another challenge relates to classification of MMR variants. Standard methodologies for variant classification differ in the classification of germline and somatic variants. Laboratories that perform germline testing rely on the correlation of predicted pathogenicity of an observed variant with the known disease phenotype (Richards et al, 2015). Interpretation of somatic sequence variants relate to their suspected impact on disease treatment and not necessarily disease development (Li et al., 2017). Therefore, a MMR variant detected by tumor genomic profiling may be classified as pathogenic, but this classification may deviate from a commercial germline report if the variant is has not been correlated with disease phenotype or if the variant has been identified in the germline previously but has not tracked with cancer risk in families.

Germline analysis

Prior to NGS and multi-gene panel testing, germline testing for LS involved Sanger sequencing with or without deletion/duplication studies for the four MMR genes and deletion testing for *EPCAM* when no tumor IHC data was available and/or a tumor

was identified to be MSI-H. When IHC results were available, genetic testing was typically offered for one or two genes that matched with the IHC results. The LS genetic testing paradigm has shifted to testing for all five genes, regardless of the clinical scenario, either alone or in combination with other hereditary colon, uterine and/or ovarian cancer genes. This shift was primarily driven by NGS lowering the cost of genetic testing. Other factors leading to adoption of multi-gene panel testing for LS include other genes being identified that may cause a dMMR tumor (Elsayed et al., 2015; Heitzer & Tomlinson, 2014), the possibility of confirming LS and identifying a second hereditary cancer syndrome (LaDuca et al., 2014; Susswein et al., 2016), and the personal and/or family history raising multiple hereditary cancer syndromes in the differential diagnosis.

When evaluating an individual for LS, deciding when to take a single/multiple gene, a multi-gene panel, or a combined somatic and germline approach will be dictated by the clinical scenario as well as the patient and/or healthcare provider preference. For individuals diagnosed with CRC under the age of 50, Pearlman et al. identified 16% with a hereditary cancer syndrome, 8% being due to LS and the other 8% caused by other high and moderate risk genes (Pearlman et al., 2017). Yurgelun et al. tested a series of unselected individuals with CRC and found ~10% had a hereditary cancer syndrome, including 3% being attributed to LS (Yurgelun et al., 2017). In a situation in which there is an affected individual with dMMR tumor results and a family history suggestive of LS, starting with a single gene(s) may make the most sense as the yield for LS is going to be high (~80%) (Pearlman et al., 2017). If the family history is not suggestive of LS or the individual was diagnosed at an older age, a combined somatic and germline approach may be best to assess LS versus biallelic somatic MMR inactivation. If an affected individual has a pMMR tumor but a strong family history indicative of LS or a young age of diagnosis, a multi-gene panel approach including all LS genes and genes associated with other syndromes may be the better option in the event the patient has another syndrome mimicking LS in presentation (Box 6) (e.g., *POLE*, *PTEN*, biallelic *MUTYH*, etc.). In addition to these options, taking a stepwise or reflex approach is an option when a single gene(s) is assessed first, but if the results are negative, moving to a multi-gene panel or a combined somatic/germline approach

can be considered. A stepwise approach can help minimize the risk for inconclusive test results. Unaffected individuals who are referred for a genetics evaluation for LS can be offered a multi-gene panel if an affected individual is not available for testing (NCCN, 2021). If the results are normal, it may be worth discussing the option of securing a tumor block or banked DNA from an affected relative for additional testing or attempting to test another relative to see if the negative results can be clarified further. Aside from clinical scenarios, other factors that dictate genetic testing approaches can include: patient interest, insurance coverage, patient health, distance to the clinic, lack of other family members, healthcare provider preference, and institution or clinic protocol.

Other genes and syndromes associated with MMR deficiency

Other germline pathogenic/likely pathogenic variants may result in dMMR tumors. Biallelic *MSH3* pathogenic/likely pathogenic variants are associated with a recessive subtype of attenuated colonic polyposis, in addition to an increased risk of dMMR CRC (Adam et al., 2016). Germline pathogenic/likely pathogenic variants in *POLE* and *POLD1* are associated with an autosomal dominant condition that can cause dMMR CRC and EC (Bellido et al., 2016; Valle et al., 2014) as well as other cancer types and gastrointestinal polyposis. MAP predisposes to a high lifetime risk of CRC that may arise through the MSI pathway (Colebatch et al., 2006). One of the benefits of multi-gene panel testing is the ability to evaluate for multiple genes concurrently. This is especially beneficial when various differential diagnoses may be possible for the presenting personal and/or family history. Given the growing list of etiologies for dMMR CRC and other tumors associated with LS, multi-gene panel testing is a strong consideration when genetic testing is indicated in dMMR tumors (Box 6).

Cascade Testing

Cascade testing is the process of testing at-risk relatives for previously identified germline pathogenic/likely pathogenic variant(s). Cascade testing for LS is a tier-one genomic application by the Center for Disease Control's Office of Public Health Genomics (Box 6) (Roberts et al., 2018). Additionally, the EGAPP working group recommended LS tumor screening for individuals with newly diagnosed CRC, mainly to reduce morbidity and mortality in relatives of the proband using cascade testing (EGAPP, 2009). Cascade testing has also been shown to be one of the main drivers

that influences the cost-effectiveness of universal tumor screening in LS cancers (EGAPP, 2009; Mvundara et al., 2010; Nikolaidis et al., 2018).

Recent reviews of cascade testing revealed that there is a wide range of uptake of genetic testing in relatives with LS (Menko et al., 2019; Roberts et al., 2018). When genetic centers contacted relatives of probands found to have LS, the uptake of cascade testing was 41 to 94% (Menko et al., 2019). Even though this strategy has been shown to be effective in research settings, using qualified healthcare providers to contact relatives directly, as opposed to having the proband or other family members do this, may be resource prohibitive. Additionally, there are ethical and legal concerns of this practice, especially in the US where there may be state laws that prohibit this practice (Roberts et al., 2018). It is important to note that the majority of probands are in favor of having themselves or someone else in the family inform at-risk relatives regarding the need for cascade testing (van den Heuvel et al., 2019). However, the effectiveness of this strategy is “disappointing” according to Menko and colleagues (Menko et al., 2019).

There are other strategies that may increase the recruitment of relatives for cascade testing. Using family letters, social media platforms for informing relatives, and/or re-education of probands about the importance of cascade testing, are all opportunities that may increase success of these programs. It is important that further research in this area be performed to identify the most effective tools and strategies for implementing these strategies into clinical practice.

Germline testing for minor children may be requested by some families with LS. The NSGC does not encourage genetic testing of minors for adult-onset conditions if the outcome does not impact medical care (NSGC, 2018) (Box 6). This position likely applies to the majority of LS families. However, there may be settings where testing of minor children for LS is necessary. One scenario in which this can occur is if there was a CRC diagnosed under age 22 in the family where colonoscopy should begin under age 18 (2-5 years prior to the earliest diagnosis in the family). Testing is recommended prior to the age at which management would change so in this case minors might be offered testing. Another scenario would be if both parents were carriers of a pathogenic/likely pathogenic variant in the same MMR gene and children are at 25%

risk for CMMRD, 50% risk for LS, and 25% risk for having no MMR gene pathogenic/likely pathogenic variant (please see following section). Other scenarios may include at-risk minors who have been diagnosed with a cancer where testing may be helpful for treatment (i.e. immunotherapy) or those undergoing tumor genomic profiling. Families in these situations are encouraged to meet with a genetic counsellor to discuss risks/benefits, disclosure strategies to the minor child and review implications of testing (NSGC, 2018).

Reproductive risk for CMMRD syndrome

When someone of reproductive age is found to have LS, it is recommended that they be educated regarding the risks of CMMRD so that each person can make their own decision regarding additional testing to clarify CMMRD syndrome risk in offspring (Box 6) (NCCN, 2021). This discussion may be especially important for carriers of *MSH6* or *PMS2* pathogenic/likely pathogenic variants, as these genes are the most common causes of CMMRD, and carriers may be under-recognized due to the lower penetrance of these genes. It is important to note that CMMRD is the result of biallelic mutations in the same MMR gene (except in the rare circumstance of pathogenic/likely pathogenic variants in *MSH2* and *EPCAM*). Therefore, if one partner has an *MLH1* mutation, while the other partner has a *MSH2* mutation, their offspring are not at risk to have CMMRD. If an individual and his or her partner are both found to have LS due to pathogenic/likely pathogenic variants in the same gene, education regarding prenatal diagnosis and assisted reproductive options, with regard to CMMRD, should be discussed (NCCN, 2021).

Summary

This practice resource is intended to provide guidance for performing a genetic evaluation for LS. This practice resource was not developed to replace a thorough cancer risk assessment by a qualified genetics professional. Genetic cancer risk assessment is an important component of a LS evaluation given that testing can be complex, tumor and molecular results may not be straightforward, and psychosocial issues may arise, all of which necessitate involvement of a specialized genetics professional. As the field of genetics is rapidly evolving, it is critical that all healthcare

professionals who evaluate patients for LS remain current on advances in this constantly changing field.

Author Contributions

All authors made substantial contributions to the conception or design of the work as well as significant efforts in drafting the work or revising it critically for important intellectual content. All authors agree to be accountable for all aspects of the work in insuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors provided final approval of the version to be published.

Compliance with Ethical Standards

The author group composition is in compliance with the National Society of Genetic Counselors Practice Guidelines Committee Conflict of Interest Policy. This policy requires all proposed authors to disclose all conflicts of interest (whether actual, potential, or perceived) prior to selection and imposes thresholds for conflicts of interest with the potential for direct, personal financial benefit to an author, or other actual, potential or perceived conflict of interest arising in connection with the development or publication of an NSGC PR.

Conflict of Interest Statement

Mr. Jasperson is a full-time salaried employee of Ambry Genetics. Ms. Hampel is on the scientific advisory boards for Invitae, Genome Medical and Promega. She has stock/options in Genome Medical and GI OnDemand. Dr. Yurgelun has research funding from Janssen Pharmaceuticals, is on the consulting/scientific advisory board for

Janssen Pharmaceuticals, and has received payment for peer review services from UpToDate. Ms. Palaniappan a full time employee of Variantyx. Dr. Hall has no financial COI to disclose. Dr. Hall has conducted collaborative research with several genetic testing entities including Myriad Genetics, Inivitae, Caris Lifesciences, Foundation Medicine, GeneDx and Ambry and has published collaborative research papers with these laboratories. No compensation or funding was received for this research.

Human Studies and Informed Consent

No human studies were carried out by the authors for this article.

Animal Studies

No animal studies were carried out by the authors for this article

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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BOX 1: POINTS TO CONSIDER - NOMENCLATURE

- The term Lynch syndrome should only be used for individuals identified with germline heterozygous pathogenic/likely pathogenic variants in the MMR genes *MLH1*, *MSH2*, *MSH6* or *PMS2* or 3' terminal deletions of *EPCAM*.
- The terms "Turcot syndrome" and "Muir Torre syndrome" are outdated and should not be used. Any patient with LS can develop a sebaceous skin lesion or a glioblastoma multiforme.
- Any label utilizing the term "Lynch syndrome" or implying an inherited aspect to dMMR tumors, in the absence of germline MMR pathogenic/likely pathogenic variants, should be avoided.
- The term constitutional mismatch repair deficiency (CMMRD) describes the biallelic inheritance of germline MMR pathogenic/likely pathogenic variants.

BOX 2: POINTS TO CONSIDER – CLINCIAL FEATURES

- Increases in tumor and germline testing are identifying more individuals and families with LS, which is redefining penetrance estimates. These estimates will continue to evolve as will the LS-tumor spectrum.
- Penetrance estimates for LS vary by gene and sex.
- There may be increased risks for other types of cancers in LS that have yet to be confirmed.

BOX 3: POINTS TO CONSIDER – CLINICAL TESTING CRITERIA

Clinical Criteria for Identifying Individuals Who Should be Evaluated for Lynch Syndrome

- Family history of a known germline MMR pathogenic/likely pathogenic variant
- Personal history of CRC or EC with any of the following characteristics:
  - Age of diagnosis <50 years
  - Tumor is dMMR: MSI-high or abnormal MMR IHC
  - Another LS-related cancer*
  - Family history of LS-related cancers in first- or second-degree relatives
    - ≥1 relative(s) diagnosed at age <50
    - ≥2 relatives diagnosed at any age
- Family history of cancer meeting any of the following criteria
  - ≥1 first-degree relative(s) with CRC or EC diagnosed age <50
  - ≥1 first-degree relative(s) with >1 diagnoses of LS-related cancers
  - ≥2 or more first- or second-degree relatives with LS-related cancers with ≥1 diagnosed age <50
  - ≥3 or more relatives with LS-related cancers at any age
- Genetic risk model score ≥5% predicted probability of germline MMR pathogenic/likely pathogenic variant (e.g. PREMM5, MMRpro)

*LS cancers: colorectal, endometrial, small bowel, urothelial, ovarian, stomach, biliary, pancreatic, sebaceous, brain

BOX 4: POINTS TO CONSIDER – TUMOR TESTING

- See Figure 1 for a simplified overview of LS evaluation
- Universal tumor screening for LS is recommended for all individuals with CRC or EC regardless of age.
- Either MSI or MMR IHC can be used to screen tumors for dMMR for universal tumor screening. For families highly suspicious for LS; testing for both MSI and MMR IHC should be considered.
- MSI and MMR IHC can be applied to any cancer type, regardless of inclusion in the LS-tumor spectrum. Sensitivity and specificity in diagnosing LS is dependent on cancer type.
- CRC shown to have dMMR, either MSI-H or MLH1 deficient, should have *MLH1* promoter hypermethylation ruled out by *BRAF* mutation testing and/or *MLH1* methylation testing.
- EC shown to have dMMR, either MSI-H or MLH1 deficient, should have *MLH1* promoter hypermethylation ruled out by *MLH1* methylation testing. *BRAF* mutation testing is not applicable for EC.
- Patients with dMMR tumors, of any kind, and no *MLH1* promoter hypermethylation should be offered germline genetic testing.
- Constitutional *MLH1* promoter hypermethylation testing should be considered in patients with tumors diagnosed at an early age, multiple primary tumors showing *MLH1* promoter hypermethylation, tumors showing *MLH1* hypermethylation in both the tumor and normal tissue, negative for a germline pathogenic/likely pathogenic variant, or clinically suspected to have LS.
- Biallelic somatic MMR inactivation is a common cause of dMMR. Germline genetic testing for LS and tumor sequencing to rule out biallelic somatic inactivation can be done sequentially or as the initial combined test. Decisions about the best testing method may be based on the likelihood of a patient having LS or biallelic somatic inactivation based on their IHC results.

BOX 5: POINTS TO CONSIDER – TUMOR GENOMIC PROFILING

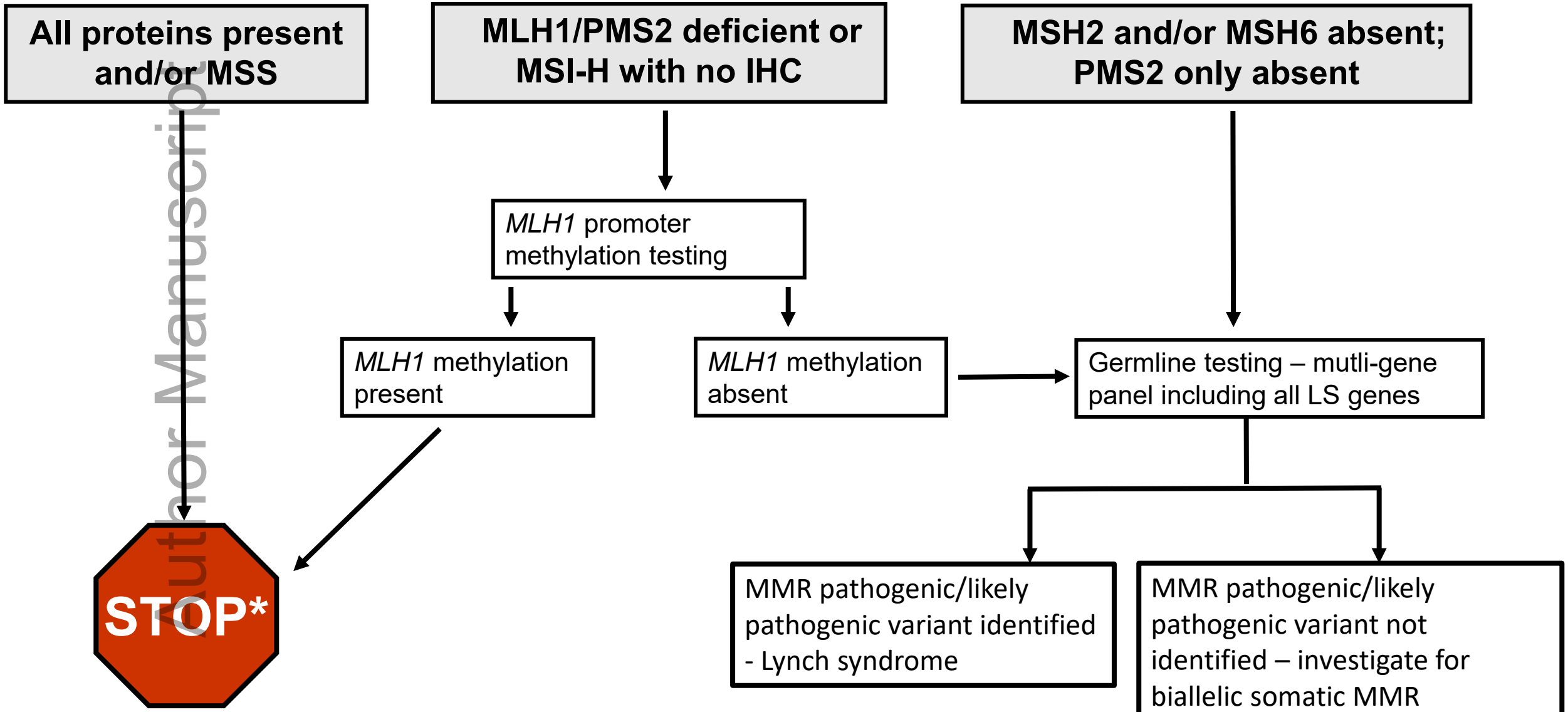
- Some variants identified on tumor genomic profiling may represent germline variants with clinically significant implications.
- All individuals with dMMR tumors should be evaluated for LS, even if the tumor is not part of the classic LS tumor spectrum, unless there is conclusive evidence of a somatic explanation for dMMR.
- Genetic counseling and confirmatory germline testing is recommended for individuals whose tumor genomic profiling identifies a pathogenic/likely pathogenic variant suspected to be germline.
- Providers should be aware of the heterogeneous nature of commercial tumor genomic profiling platforms regarding how parallel germline sequencing is handled and reported.
- Patients should informed of the potential of identifying germline pathogenic/likely pathogenic variants through genomic profiling and given the option to opt out of receiving germline results.

BOX 6: POINTS TO CONSIDER – GERMLINE TESTING

- Ordering a multi-gene panel test for the evaluation of LS is considered best practice in the age of NGS.
- Germline analysis for dMMR tumors by multi-gene panel testing should include the MMR genes as well as additional genes known to cause dMMR tumors such as *MSH3, MUTYH, POLD1,* and *POLE.*
- Cascade testing should be encouraged and facilitated for individuals and families at risk of inheriting pathogenic/likely pathogenic variants.
- Testing for LS is not advised for minor children unless results could impact immediate medical care.
- Any individual of reproductive age and/or who has not completed their family planning identified to have an MMR or *EPCAM* pathogenic/likely pathogenic variant should be counseled about the reproductive risks for CMMRD.

Figure 1. Lynch Syndrome Evaluation

This is a simplified overview of the process of LS evaluation. This could vary based on tumor type (e.g. *BRAF* testing should be included prior to MLH1 methylation in all CRCs). See full text for more detailed testing process.



*Genetics referral based on family history, early onset, or polyp numbers

Table 1. Lynch Syndrome Cancer Risks to 70 years

Cancer Type		General Population	*MLH1*	*MSH2*	*MSH6*	*PMS2*
Colorectal	Male	4.41	41-67%	43-55%	12-31%	12-20%
	Female	4.08	35-61%	47-68%	12-30%	12%
Endometrial	Male	-	-	-	-	-
	Female	3.07	19-57%	21-57%	16-46%	13-15%
Ovarian	Male	-	-	-	-	-
	Female	1.25	10-20%	15-24%	10-13%	3%
Gastric	Both Genders	0.66-1.07	5-7%	0.2-16%	0-5%	-
Ureter and Kidney	Male	2.16	1-14%	5-20%	1-3%	-
	Female	1.23	0.7-4%	5-19%	0-6%	-
Bladder	Male	3.86	6-11%	12-21%	2-21%	-
	Female	1.18	0-5%	2-12%	0-2%	-
Small Bowel	Both Genders	0.00	0.5-11%	1-10%	0-3%	-
CNS	Male	0.69	1-2%	7-8%	1-2%	-
	Female	0.55	1-2%	2-3%	1-2%	1-2%
Prostate	Male	11.60	13-17%	23-32%	0-9%	4-5%
	Female	-	-	-	-	-
Breast	Male	0.13	-	-	-	-
	Female	12.83	12-13%	14-15%	13-14%	15-16%

Baglietto et al., 2010; Bonadona et al., 2011; Choi et al., 2009; Dominguez-Valentin et al., 2020; Dominguez-Valentin et al., 2021; Engel et al., 2012; Haraldsdottir et al., 2014; Moller et al., 2017; NCCN, 2021; Ramsoekh et al., 2009; Roberts et al., 2018; Ryan et al., 2014; Senter et al., 2008; Stoll et al., 2020; ten Broeke et al., 2018; Therkildsen et al., 2017; van der Post et al., 2010; Vasen et al., 2001; Win et al., 2021

Table 2. Colorectal Cancer Causes of dMMR by IHC/MSI Finding

IHC Result	n	Lynch syndrome	Bialleic Somatic	Unexplained	False-positive IHC
Absent MLH1/PMS2*	75	28 (37.3%)	45 (60%)	1 (1.3%)	1 (1.3%)
Absent MSH2/MSH6	80	55 (68.8%)	18 (22.5%)	6 (7.5%)	1 (1.3%)
Absent MSH6	33	19 (57.6%)	5 (15.2%)	3 (9.1%)	6 (18.2%)
Absent PMS2	29	21 (72.4%)	5 (17.2%)	1 (3.5%)	2 (6.9%)
IHC normal (MSI-H)	17	11 (64.7%)	3 (17.6%)	3 (17.6%)	0
Total	232	134 (57.8%)	76 (32.8%)	14 (6%)	10 (4.3%)

*MLH1 methylation was ruled out first in cases with absence of MLH1 and PMS2 because MLH1 promoter methylation is the most likely cause of this IHC finding.

Adapted from Pearlman et al. (2019)