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Lynch Syndrome Genetics and Clinical Implications




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Lynch syndrome (LS) is one of the most prevalent hereditary cancer syndromes in humans and accounts for some 3% of unselected patients with colorectal or endometrial cancer and 10%–15% of those with DNA mismatch repair–deficient tumors. Previous studies have established the genetic basis of LS predisposition, but there have been significant advances recently in the understanding of the molecular pathogenesis of LS tumors, which has important implications in clinical management. At the same time, immunotherapy has revolutionized the treatment of advanced cancers with DNA mismatch repair defects. We aim to review the recent progress in the LS field and discuss how the accumulating epidemiologic, clinical, and molecular information has contributed to a more accurate and complete picture of LS, resulting in genotype- and immunologic subtype–specific strategies for surveillance, cancer prevention, and treatment.

Keywords: Lynch Syndrome; Colorectal Cancer; Endometrial Cancer; DNA Mismatch Repair; Genetic Testing; Cancer Prevention.

Lynch syndrome (LS) represents an autosomal dominant predisposition to colorectal carcinoma (CRC), endometrial carcinoma (EC), and other cancers because of defective DNA mismatch repair (dMMR). The history of the syndrome dates back to 1895, when Dr Warthin started to collect information on the first LS family later, designated Family G.[1] The stringent Amsterdam criteria,[2,3] which require 3 or more family members diagnosed with an LS-associated cancer at an early age, were formulated to guide the selection of families for molecular studies. These and the less stringent Bethesda criteria[4] facilitate recognition of LS in the clinical setting. The discovery of the 4 LS-associated DNA mismatch repair (MMR) genes—*MSH2*, *MLH1*, *MSH6*, and *PMS2*—in 1993–1995 marks the beginning of the molecular era of LS.[5] Definitive diagnosis of LS requires the identification of a pathogenic or likely pathogenic constitutional variant affecting one of the MMR genes (or *EPCAM*), and the term LS is currently restricted to cases fulfilling this molecular definition.

Prevalence

dMMR often results in absent MMR protein(s) and microsatellite instability (MSI) in tumor tissue, providing valuable shortcuts to the identification of LS among consecutive patients with CRC or EC. This so-called universal tumor screening followed by constitutional testing has led to an estimate of some 3% of CRCs[6] and a roughly similar proportion of ECs[7] being attributable to LS. Recent studies applying hereditary cancer panel testing to patients with unselected CRC[8] or EC[9] without prior tumor-based screening arrived at estimates of 3% and 6% of LS among all CRCs and ECs, respectively. Using nuclear families of nearly 6000 incident CRC cases recruited irrespective of family history from population-based cancer registries, Win et al[10] estimated that 1 in 279 individuals (0.359%) could be carriers of pathogenic variants of any MMR gene in the US, Canadian, and Australian populations. In a gene-specific analysis, *PMS2* and *MSH6* were associated with the highest population prevalences, 1 in 714 (0.140%) and 1 in 758 (0.132%), respectively, compared with *MLH1* (1 in 1946 [0.051%]) and *MSH2* (1 in 2841 [0.035%]).[10] The finding reflects lower penetrance of *PMS2* and *MSH6* compared to *MLH1* and *MSH2*. Accordingly, enrichment of founder

Abbreviations used in this paper: **CMMRD, constitutional mismatch repair deficiency; CRC, colorectal carcinoma; dMMR, defective DNA mismatch repair; EC, endometrial cancer; FCCTX, familial colorectal cancer, type X; h, human; LLS, Lynch-like syndrome; LS, Lynch syndrome; MMR, DNA mismatch repair; MSI, microsatellite instability; MSI-H, microsatellite instability of high degree; MSS, microsatellite stable; PLSD, Prospective Lynch Syndrome Database; pMMR, mismatch repair proficiency/proficient.**

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variants in *PMS2* and *MSH6* resulted in a high LS carrier frequency (1 in 226 [0.442%]) in the Icelandic population.[11] Population frequencies of even up to 1 in 100 have been suggested.[12] These figures make LS the most common form of hereditary CRC and probably the most prevalent single-gene cause of cancer predisposition overall.

Clinical Phenotypes

Phenotypes associated with constitutional variants of MMR genes depend on heterozygosity vs homozygosity for the predisposing defects (Table 1). Heterozygous variants of *MLH1*, *MSH2*, *MSH6*, and *PMS2* underlie LS predisposition.[13,14] Rarely, genomic deletions of the 3′ end of *EPCAM* may cause LS predisposition through epigenetic inactivation of a structurally intact *MSH2*.[15] Muir–Torre syndrome may accompany LS.[16] LS also covers part of Turcot syndrome[17] as a phenotypic variant (Table 1). In rare instances (∼200 cases reported), pathogenic constitutional variants in any 1 of the 4 LS-associated MMR genes (or 3′ untranslated region deletions of *EPCAM*) may occur at a homozygous or compound heterozygous state. This results in a distinct syndrome called constitutional mismatch repair deficiency (CMMRD).[18–20] The predominant genes underlying CMMRD are *PMS2* and *MSH6*, possibly reflecting higher population prevalence and lower penetrance (better tolerability) of their variants compared to *MLH1* and *MSH2*.[18]

Lynch Syndrome Genes and Functions of DNA Mismatch Repair Proteins

DNA Mismatch Repair Mechanism

The primary responsibility of the MMR system is to correct errors that arise during DNA replication and recombination—a function critical for cancer avoidance.[21] In humans, 5 MutS homologues (MSH2, MSH6, MSH3, MSH4, and MSH5) and 4 MutL homologues (MLH1, PMS2, PMS1, and MLH3) exist, 6 of which function in MMR (Figure 1). The main mismatch-binding factor in humans (h) is hMuSα, a heterodimer of MSH2 and MSH6. Another mismatch-recognition complex is hMutSβ formed by MSH2 and MSH3. MSH6 is required for the correction of single base mispairs and 1–2 nucleotide insertion–deletion loops, and both MSH3 and MSH6 may participate in the correction of insertion–deletion loops larger than 2 nucleotides. The MSH proteins are in the resting state in the adenosine diphosphate–bound form. Adenosine triphosphate–bound hMutSα or hMutSβ undergoes a conformational change into a clamp that moves along the DNA to signal to the additional components of the MMR machinery.[22] MLH1 and PMS2 (the latter is a homologue of yeast PMS1) form hMutLα, which coordinates the interplay between the mismatch-recognition complex and other proteins necessary for MMR (Figure 1). Although hMutLα is the main hMutL heterodimer, MLH1 can also complex with MLH3 (hMutLγ) and PMS1 (hMutLβ). PMS2 is primarily required for the correction of single base mispairs, whereas MLH3 may contribute to the repair of insertion–deletion loops and, additionally, the correction of mismatches if PMS2 is absent.[23] The hMutLβ complex does not seem to participate in MMR. Unlike *Escherichia coli*, whose MMR is methyl directed (a transient lack of methylation identifies the nascent strand), DNA replication-associated daughter strand nicks that direct asymmetric loading of proliferating cell nuclear antigen likely mediate strand discrimination in eukaryotes.[24] Upon encountering a strand discontinuity, the hMutS–hMutL complex recruits excision machinery, and

Table 1. LS and Related Phenotypes

Syndrome	MIM number[a]	Susceptibility genes	Mode of inheritance	Clinical features
LS[b]	609310 120435 614350 614337 185535	*MLH1* (41%) *MSH2* (36%) *MSH6* (18%) *PMS2* (5%) *EPCAM* (rare)	AD	Colonic and extracolonic cancers of a defined spectrum[3] and occurring earlier than in the average population (at ∼40–60 years of age)
Muir–Torre syndrome	158320	Mostly *MSH2* and *MLH1*	AD	Multiple sebaceous gland adenomas co-occurring with visceral malignancies, such as colorectal carcinoma
Turcot syndrome	—	See LS and CMMRD	AD or AR[c]	Primary brain tumors co-occurring with multiple colorectal adenomas
CMMRD	276300	Mostly *PMS2* and *MSH6*	AR	Childhood cancers, mainly hematologic malignancies and/or brain tumors, signs of neurofibromatosis type 1 (café-au-lait spots), combined with early-onset colorectal cancers and polyposis

AD, autosomal dominant; AR, autosomal recessive; MIM, Mendelian Inheritance in Man.
[a]Phenotype number in MIM.[14]
[b]Shares of *MLH1*, *MSH2*, *MSH6*, and *PMS2* variants are based on a total of 2105 variants of classes 3–5 reported in Thompson et al.[13]
[c]Turcot syndrome may arise as a variant of LS (dominant) or CMMRD (recessive). It may also arise from constitutional defects of *APC* (MIM no. 175100), in which case the transmission pattern is dominant.

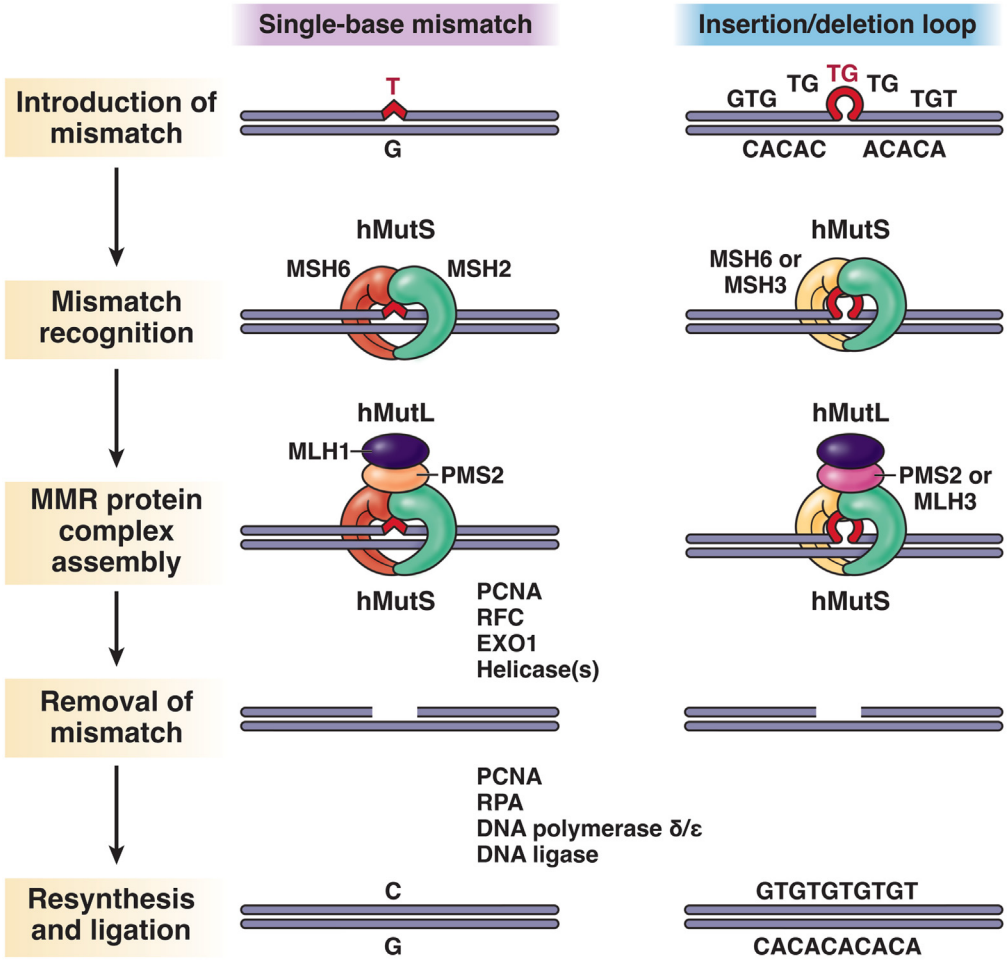

Figure 1. Function of the human MMR system in the correction of single-base mismatches (G to T, *left*) and insertion–deletion loops (TG insertion, *right*) that have arisen as replication errors in the newly synthesized strand (*red*). See text for details.

degradation of the error-containing fragment and synthesis of a new strand follow.

Microsatellite Instability as a Hallmark of Lynch Syndrome

Defective MMR results in length variation of short tandem nucleotide repeats, microsatellites (MSI). MSI is a hallmark of LS and up to 30% of sporadic cancers of various organs.[25] Different substrate preferences of individual MMR proteins may explain different MSI phenotypes resulting from MMR gene defects.[26] *MSH2*, *MLH1*, or *PMS2* inactivation is associated with high-degree MSI (MSI-H) with mononucleotide, dinucleotide, and other short tandem repeats affected. *MSH6* inactivation mainly results in mononucleotide repeat instability. *MSH3* (hMutSβ) dysfunction may lead to a distinct form of MSI called elevated microsatellite alterations at selected tetranucleotide repeats, also seen in tumors from carriers of biallelic *MSH3* constitutional defects.[27] Whether *MLH3* inactivation results in a specific type of MSI or not is unclear. Tumors from biallelic *MLH3* variant carriers showed no instability at mono-, di-, tri-, or tetranucleotide repeats.[28]

Other Functions of DNA Mismatch Repair Proteins

If correction of replication errors is not possible, MMR proteins signal DNA damage to cell cycle arrest or apoptosis.[29] The MMR system also blocks recombination between related but nonidentical (homeologous) sequences, acting as a barrier to chromosomal rearrangements.[30] Under certain circumstances, MMR proteins can promote sequence alterations. Inferred from yeast studies, the MLH1–MLH3 complex (hMutLγ) and the MSH4–MSH5 complex (hMutSγ) facilitate meiotic crossover between homologous chromosomes.[31] PMS1 (yeast MLH2), too, functions in meiosis. The MLH1–PMS1 complex (hMutLβ) limits the length of the gene conversion tract in meiotic recombination.[31] When the MMR system recognizes mismatches outside replication, strand discrimination between the old and new DNA is lost, and the MMR proteins can act

mutagenically and contribute to trinucleotide repeat expansion.[32,33] Counterintuitively, trinucleotide repeat expansion requires MMR proficiency (pMMR).

Constitutional Defects Underlying Lynch Syndrome Predisposition

Genetic Alterations

MLH1 and *MSH2* are the most important predisposing genes for LS (Table 1), which is compatible with the fact that their products are obligatory components in all types of MMR protein heterodimers, whereas MSH6 is redundant with MSH3 and PMS2 is redundant with MLH3 (Figure 1). There is no convincing evidence that heterozygous variants of *MSH3* or *MLH3* would underlie LS predisposition. Interestingly, homozygous variants of these genes cause susceptibility to adenomatous polyposis with possible features of CMMRD, highlighting the dosage dependency of phenotypes associated with MMR gene defects.[27,28] There are no reports of LS-associated constitutional defects of *PMS1*, *MSH4*, or *MSH5*, which agrees with the primary role of these genes in meiotic recombination rather than MMR.

A functional MMR system needs to produce MMR proteins, transport them to the nucleus, form appropriate protein complexes at the site of the DNA mismatch, and perform the actual MMR. LS-predisposing MMR gene alterations are pathogenic through the loss of any one of these functions (often several of them), which typically results from nonsense or frameshift changes. The share of missense alterations that lead to single amino acid substitutions is also significant (30%–60%) for all 4 LS-associated MMR genes.[34] Nonsense and frameshift alterations, canonical splice site changes, and deletions of a single exon or multiple exons generally disrupt gene function and are therefore pathogenic. Although multiple in silico tools exist to predict the pathogenicity of missense alterations,[35] verification by laboratory assays,[36,37] and including tests for aberrant splicing,[38] is necessary. Characteristics of a sequence variant combined with clinical and family features have led to a 5-tiered classification[13,39] to interpret sequence variants of disease-associated genes for clinical purposes. Variants belonging to class 1 (benign) or 2 (likely benign) are considered harmless and require no special attention. Class 3 is for variants of uncertain significance, and clinical management is case by case. Class 4 (likely pathogenic) or 5 (pathogenic) indicates that the variant is deleterious, warranting surveillance according to high-risk guidelines and enabling predictive testing of at-risk relatives.

LS individuals typically inherit their predisposing variants from one of their parents, and de novo alterations are rare (2.3%).[40] Ancestral founding changes predominate in some populations and account for more than half of all LS families.[41] The extent of haplotype conservation in carriers of founding changes provides a tool to estimate the age of such alterations. Thus, a 3.5-kb genomic deletion of *MLH1* exon 16 unique to Finnish LS families started to spread 400–1075 years ago.[42] A 20-kb deletion in *MSH2* exons 1–6 characteristic of North American LS families may be 500 years old.[43] These 2 founder changes additionally illustrate richness of the MMR gene regions in Alu- and other repeats. Recombination events mediated by such repeats explain why some 10%–20% of all LS-associated changes are large genomic rearrangements.[44] All *EPCAM* alterations responsible for LS predisposition consist of large genomic deletions of the 3′ end of the gene leading to the removal of the stop codon, and Alu-mediated recombination plays a major role in their origin.[45]

Constitutional Epimutations

Constitutional hypermethylation at the promoter of one allele of *MLH1* or *MSH2* can lead to silencing of expression from that allele in all main somatic tissues, causing susceptibility to colorectal and extracolonic cancers typical of LS. Epimutation can be primary (no apparent cause for hypermethylation identifiable) or secondary (induced by genetic alteration). Primary and secondary epimutations of *MLH1* may account for 1%–10% of unexplained Lynch-suspected families with silenced MLH1 expression in tumors.[46–49] Constitutional *MLH1* epimutations are rare among unselected CRC patients.[50] Secondary epimutation caused by deletions of the 3′ end of the upstream *EPCAM* gene is the only known type of constitutional epimutation for *MSH2*. After removal of the stop codon, transcription of *EPCAM* reads into the adjacent, structurally normal *MSH2* gene, inducing its promoter methylation.[15] Secondary epimutations of *MSH2* may be responsible for a variable percentage of unexplained Lynch-suspected families (0%–40% depending on possible founder effects).[15,47] There are no reports of LS-associated constitutional epimutations for MMR genes other than *MLH1* and *MSH2*.[51]

Epigenetic changes are subject to erasure when they pass through the germline. Therefore, primary constitutional epimutations segregate in a nonmendelian fashion and are seldom associated with any remarkable family history of cancer. Families of primary epimutation carriers may exhibit mosaic epigenetic inheritance, reversion of the methylated allele to the normal active state, or apparent heritability.[52–55] Variability in transmission patterns requires appropriate consideration in genetic counseling. In contrast, secondary epimutations of *MLH1* or *MSH2* give rise to classical LS families and cosegregate with their *cis*-acting genetic changes as dominant mendelian traits.[15,56] Nevertheless, the basic mechanism of transmission may differ from that of a genetic alteration. Hitchins et al[57] showed that *MLH1* c.-27C>A–associated secondary epimutation underwent erasure in spermatozoa, followed by reestablishment in somatic cells of the next generation.

No Constitutional Defect Found—Phenotypic Confounders

Neither fulfilment of the clinical (Amsterdam) criteria nor dMMR in tumor tissue is specific for LS. Table 2 summarizes some key characteristics of LS and the various entities that mimic it. He MMR status of tumor tissues divides families meeting the Amsterdam criteria into 2

Table 2. Differential Diagnosis of LS

Clinicopathologic characteristic	LS	FCCTX	LLS	*MLH1* Methylated
Share of all CRCs, %	1–3	~1?	2–3	10–20
Average age at cancer onset, *y*	45	50–60	60	75
Tumor spectrum	Colonic and extracolonic cancers	Mainly site-specific CRC	CRC[a]	CRC[a]
Preferential location of CRC	Proximal	Distal	Variable	Proximal
Transmission pattern	Autosomal dominant	Autosomal dominant	None (some are familial)[b]	None (sporadic)
Predisposing genes	*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*	Mostly unknown, candidate genes exist[c]	None?	None
Tumor characteristics	MMR deficient and hypermutant *BRAF* V600E negative CIMP may be present	MMR proficient and mostly nonhypermutant *BRAF* V600E unknown Global hypomethylation present	MMR deficient and hypermutant *BRAF* V600E negative CIMP often present	MMR deficient and hypermutant *BRAF* V600E positive (mostly) CIMP present

CIMP, CpG island methylator phenotype.
[a]LLS and *MLH1*-methylated subgroups also exist among ECs and several other cancers.
[b]Family history of cancer may occur; the subgroup with double somatic MMR gene variants is sporadic.
[c]*GALNT12*, *RPS20*, *BRF1*, *FAN1*, *FAF1*, *SEMA4A*, and other candidate genes have been proposed.[59]

subcategories: dMMR (LS) and pMMR (familial colorectal cancer type X [FCCTX]).[58] Recent genome-wide studies have identified several putative candidate genes for FCCTX,[59] but each accounts for only a small proportion of families, and the genetic basis of FCCTX remains largely unknown, as "type X" implies. Acquired *MLH1* promoter methylation is by far the most frequent cause of dMMR in CRC (10%–20 %), whereas LS accounts for 1%–3% of all CRCs (Table 2). MMR-defective cancers that neither *MLH1* methylation nor pathogenic constitutional variants of MMR genes explain represent Lynch-like syndrome (LLS). The share of all CRCs that LLS is responsible for is comparable to LS.[60,61] Double somatic events consisting of pathogenic sequence variants of *MLH1*, *MSH2*, *MSH6*, or *PMS2* or loss of heterozygosity are detectable in more than half of LLS tumors.[61–63] It is reasonable to treat the double somatic subgroup as sporadic CRC for their heritability. However, a proportion of LLS cases displays a family history for LS-associated cancers, the basis of which is unknown.[60,64] Although certain clinicopathologic differences between LS and its phenotypic confounders are evident (Table 2), a reliable distinction between the individual conditions is achievable only by molecular methods.

Tumorigenesis in Lynch Syndrome

Two-Hit Paradigm and Haploinsufficiency

The LS genotype is heritable in an autosomal dominant pattern. A carrier of a pathogenic constitutional variant is at increased risk of cancer, but the penetrance of the phenotype is not 100%. The loss of ability to repair DNA mismatches requires, in accordance with Knudson's 2-hit hypothesis,[65] that a somatic second hit disable the functional allele of the MMR gene. Even in the event of the predisposing MMR gene retaining its wild-type allele in somatic cells, the total amount of gene product may not be sufficient for normal function (haploinsufficiency). Heterozygous *MLH1* and *MSH2* transgenic mice display decreased expression of MMR proteins[66] and increased levels of genomic frameshift deletions in the colon.[67] Reduced dose of effective MMR molecules may be tissue specific and explain some of the phenotypic variation (eg, age at disease onset) characteristic of LS.[66] Moreover, different functions may require different amounts of MMR protein. For example, DNA damage signaling requires a higher dosage of MLH1 than DNA MMR.[68] As another example, decreased messenger RNA expression of *Mlh1* and other chromosomal segregation genes in normal colonic mucosa of $Mlh1^{+/-}$ and $Mlh1^{+/+}$ mice is associated with predisposition to pMMR, chromosomally unstable CRC.[69]

Developmental Pathways and Dependence on Somatic Alterations

Normal bowel mucosa[70] and normal endometrium[71,72] of pathogenic MMR variant carriers contain dMMR niches. The role of these dMMR crypt foci has been studied more in the colorectum, but it remains unclear if they may give rise to neoplasia or not. Whole-genome sequencing analysis by Lee et al[73] showed that the vast majority of histologically normal epithelial crypts from LS individuals are genomically

stable; however, a dMMR crypt with an elevated mutation burden and dMMR-associated signature was identified that could represent a very early stage of LS colorectal tumorigenesis. Together with a well-established ineffectiveness of even the most meticulous colonoscopy surveillance to prevent colorectal cancers[74,75] rather than improve survival,[76] dMMR crypts have raised several hypotheses for unsuccessful cancer prevention by endoscopic removal of preceding adenomas.[77] The proposed mechanisms for the unsuccessful prevention despite successful colonoscopies include an evolutionary model depicted in Figure 2. Some of the cancers arising from dMMR crypts may develop as flat lesions not detectable or removable endoscopically early enough as opposed to the traditional adenoma–carcinoma sequence with a distinct temporal order of somatic hallmark variants.[78]

Lesions escaping colonoscopy surveillance may more often be associated with *MLH1* pathogenic variants, because adenomas are clearly more frequently detectable in *MSH2* carriers despite the similarly high CRC incidence.[79] Somatic *APC* variants are more common in MSH2 than MLH1-deficient cancers, whereas *CTNNB1* variants predominate in MLH1-deficient tumors.[79] Moreover, developmental pathways may differ between screen-detected and non–screen-detected CRCs. This is plausible because incident cancers detected in regular colonoscopy surveillance rarely show *KRAS* codon 12 or 13 changes, and sequence alterations of *APC* have developed downstream of MMR deficiency based on mutational signatures.[80]

An initial hypothesis postulated that *CTNNB1* variants are involved in nonpolypous carcinogenesis.[81] A study comparing PMS2- and MLH1-deficient CRCs detected no somatic *CTNNB1* variants (0/20 [0%]) in *PMS2*-associated CRCs, whereas *MLH1*-associated CRCs carried a significant number of *CTNNB1* variants (14/24 [58%]). Moreover, *KRAS* alterations appeared to precede PMS2 deficiency in adenomas and/or CRCs from *PMS2* variant carriers.[82] This seems to fit well with the clinical and epidemiologic observation that no *PMS2*-associated early-onset CRCs are encountered during colonoscopy surveillance[83] and suggests that the dMMR crypt foci pathway does not play a role in *PMS2*-defective carcinogenesis.[84]

Neoantigens and Immunogenicity

LS-associated cancers are very immunogenic and labeled as "hot" tumors that the immunomodulative therapeutics cure more frequently than tumors with lower immune cell density.[85] Abundance of effector and memory T cells in the tumor microenvironment both in the tumor core and in the invasive front presumably results from the hypermutated genomic profile. MSI-H tumors usually present with sequence alteration burden above the indicative threshold of 10 changes per megabase in sequencing.

There is more to it, however. Although it is not entirely clear if acetylic salicylate modulates the immune environment on normal bowel mucosa, preventively administered acetylic salicylate 600 mg per day for 2–4 years reduced the

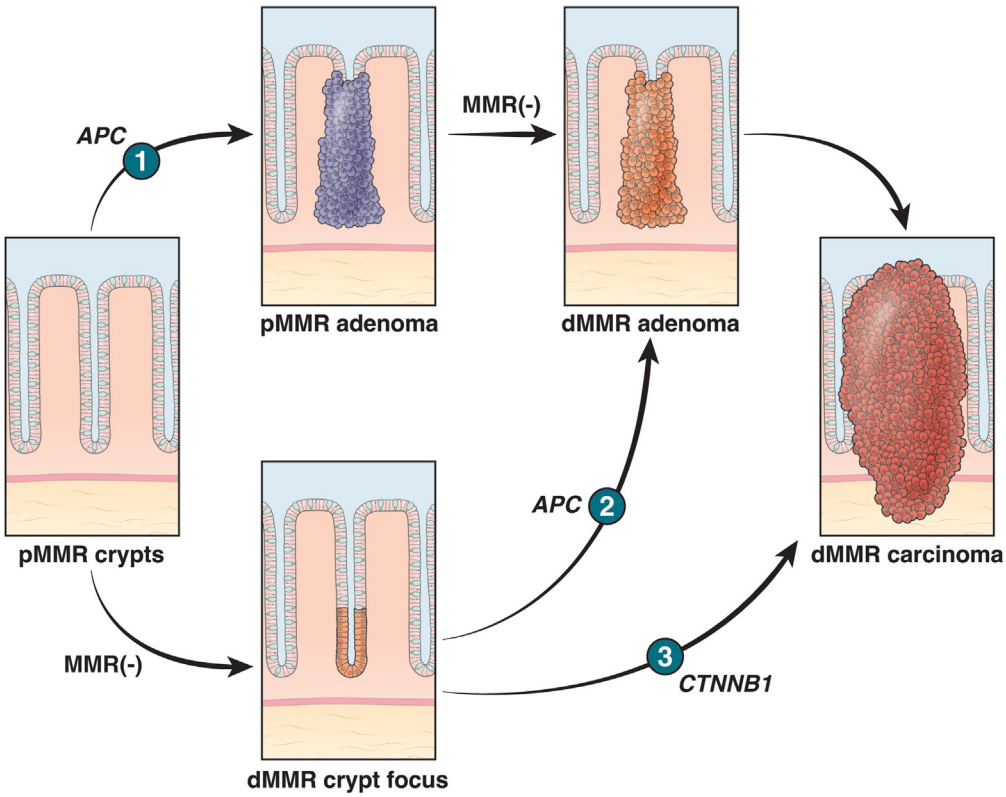

Figure 2. Alternative pathways to CRC in LS.[78,79] LS CRC may develop through pMMR adenoma with secondary MMR inactivation (pathway 1) or from dMMR crypts with or without adenoma formation (pathways 2 and 3, respectively). Pathway 2 involves formation of a polypoid lesion after *APC* inactivation and is associated with *MSH2* pathogenic germline variants. *CTNNB1* activation triggers an "immediate invasive" (pathway 3), which is a feature of CRCs from *MLH1* variant carriers.

incidence of CRC almost by half in more than 10-year follow-up[86] with a number needed to treat of 24. This double-blind randomized clinical trial showed a delayed onset of prevention, suggesting a slow onset with a lengthy legacy effect. Furthermore, either 220 mg or 440 mg of naproxen per day for 6 months induced significant molecular changes in patient-derived and mouse models of normal bowel mucosa of pathogenic MMR variant carriers. Naproxen activated different resident immune cells, reduced prostaglandin E2 levels, and promoted down-regulation of stem cell markers and up-regulation of epithelial differentiation markers.[87] Finally, LS individuals with and without cancer have different immune profiles in their normal colorectal mucosa. Pathogenic MMR variant carriers without CRC showed elevated CD3-, FOXP3-, and CD8-positive T-cell densities compared with non-LS control individuals and LS patients with CRC. Moreover, the relative immune cell density on normal bowel seems to reduce when coming closer to the diagnosis of cancer.[88] The successes of nonsteroidal anti-inflammatory drug–based chemoprevention and immune therapy by checkpoint inhibitors indicate that the epithelial immune microenvironment is a modifiable risk factor. It may even serve as a possible risk stratification biomarker for intensified surveillance and risk reduction.

Because the microsatellites often develop on the same genomic positions in microsatellite unstable cancers regardless of which tissue type the tumor arises from, the subsequent frameshifts also take place similarly, causing truncating proteins as neopeptides to form across different tumor types. This results in immunoediting by counter-selection of cell clones with the most immunogenic frameshift peptides and depends on the HLA haplotype. This dependence is lost in tumors that have acquired a somatic β_2 microglobulin alteration that reduces counterselection.[89] The shared frameshift alterations in MSI tumors open an avenue for successful immunotherapy and immune prevention by using these peptides as neoantigens for adaptive immunity (see Seth et al[90] and Cerretelli et al[91] for overviews and the "Vaccine-Based Immunotherapy and Immunoprevention" section).

Phenotypic Correlations of Molecular Alterations

Cancer Risks Associated With Constitutional Defects of Individual DNA Mismatch Repair Genes

Large observational data recorded in the Prospective Lynch Syndrome Database (PLSD) have consistently shown that the cancer risks associated with each MMR gene differ from one another.[83,92,93] Pathogenic variants in *MLH1* and *MSH2* produce a lifetime risk of any cancer of approximately 80% despite surveillance efforts, with the median age of onset being 48–54 years with little difference between sexes. However, lifetime cancer risk in *MSH6* pathogenic variant carriers is 29% in males and 55% in females at a median age of onset of 56–57 years, with a substantial sex-limited trait because of the high risk of EC. Early-onset cancers do not generally take place in *PMS2* pathogenic variant carriers, but there is prospective observational evidence of CRC and EC at a median age of 66–70 and 61 years, respectively. *PMS2* findings from the PLSD comply with observations by Senter et al[94] of unremarkable family histories of carriers of heterozygous variants ascertained through isolated loss of PMS2 in tumor tissue.

It is unknown whether and to what extent features of the MMR mechanism, type and location of the predisposing variant, or somatic alterations directed by the constitutional defects may explain the associated phenotypic differences between the 4 main LS predisposition genes. Tissue-specific reduction in the dose of MMR protein may explain some of the variation between the functional protein deficiencies by gene (see the "Tumorigenesis in Lynch Syndrome" section). Observational data comparing truncating pathogenic *MLH1* and *MSH2* variant carriers to nontruncating variants has not demonstrated differences in lifetime penetrance of cancer.[95]

Prognostic Correlations

The major LS-associated cancer types are associated with excellent survival: 10-year crude survival was 88% for cancer of the colon, 70% for rectum, 89% for endometrium, and 84% for ovary according to the PLSD data.[83] Early detection due to increased awareness and regular surveillance, combined with inherent biological properties of LS tumors, likely contribute to the good prognosis. In CRC, MSI is a generally favorable prognostic sign.[96] LS-associated CRCs are immunologically active because of their inherent "mutator phenotype."[97] Metastatic MMR-deficient cancers from LS and other patients overexpress immune checkpoint ligands, which makes them responsive to immune checkpoint inhibitors.[98] The favorable prognosis of LS-associated ovarian carcinomas compared to those from the general population is especially striking. Underrepresentation of serous cancers in LS does not alone provide a satisfactory explanation,[99] and unique molecular[100] and immunologic[101] properties of LS ovarian tumors may serve as additional prognostic contributors.

Lynch Syndrome Tumor Spectrum

The Amsterdam II criteria[3] acknowledge cancers of the colon and rectum, endometrium, small bowel, ureter, and renal pelvis as LS-associated cancers because these are significantly more frequent in LS compared to the average population. Later studies have consistently reported significantly increased risks for additional cancers, including cancers of the stomach, ovaries, pancreas, and several other organs, in LS carriers vs the general population or non-carriers.[93,102–104] Urothelial and prostate cancers are especially associated with the MSH2 genotype.[83]

MSI and/or extinct MMR protein expression in tumor tissue provides an additional tool to evaluate if the predisposing defect contributes to tumor development. Among cancers from pathogenic MMR gene (mainly *MLH1*) variant

carriers from a nationwide registry,[34] immunohistochemical analysis regularly showed absent MMR protein(s), whereas MSI-H in the same tumor types varied from 80%–100% (stomach, ovary, colon, and ureter) to approximately 50% (bladder, endometrium, and kidney) and even less (35% for breast and 0% for brain tumors). Different growth patterns (clonal heterogeneity) may offer one possible explanation for the varying frequencies of MSI among different tumor types from LS individuals. Latham et al[105] investigated more than 15,000 tumors (over 50 cancer types) for MSI and analyzed matched constitutional DNA for alterations of MMR genes and *EPCAM* regardless of MSI status. Pathogenic constitutional variants (ie, LS) were identified in 16.3%, 1.9%, and 0.3% of patients with MSI-H, MSI-indeterminate, and microsatellite-stable (MSS) tumors, respectively. Two observations relevant to the LS tumor spectrum stand out. First, among LS patients with MSI-H (plus indeterminate) tumors, half had tumors other than CRC or EC, including tumor types not previously or regularly connected to LS, such as mesothelioma, melanoma, soft tissue sarcoma, and prostate cancer. Second, 36% of LS patients had MSS tumors, and these were predominantly non-CRC/ECs. Reduced penetrance of MMR gene variants (*MSH6*) or low tumor purity might explain MSS.[105] MSS tumors could also arise through non-MMR functions of MMR proteins (see the "Lynch Syndrome Genes and Functions of DNA Mismatch Repair Proteins" section) or be truly sporadic. Breast cancer illustrates a cancer type whose relationship to LS is controversial, but at least a subset seems etiologically linked to the predisposing MMR defects based on dMMR tumor profiles[106–108] and may respond to anti-PD1/PD-L1 immunotherapy.[108]

Detection of Lynch Syndrome Predisposition

Population Screening

Multiple guidelines recommend universal tumor screening, that is, testing any new CRC[109,110] or EC[110,111] for deficient MMR protein expression and/or MSI, to select potential cases of LS for constitutional testing. Universal screening is cost-efficient for LS identification alone,[112,113] with further major implications for overall cancer management after the checkpoint inhibition therapy became available. Although immunohistochemical analysis for MMR protein expression and MSI testing have similar sensitivities and specificities and a generally good concordance,[6,112,114,115] neither method is 100% accurate. Some LS-predisposing missense (eg, *MLH1* P28L[116] and *MSH2* T33P[117]) and even truncating (*MSH6* V131fs*2[114]) variants may give rise to stable but nonfunctional protein. Conversely, immunohistochemical analysis may show abnormal protein expression in some LS tumors that remain MSS because of, for example clonal heterogeneity or low tumor purity.

Compared to MSI testing, immunohistochemical analysis has an additional advantage of identifying the specific MMR protein with aberrant expression, thus pinpointing the gene likely altered constitutionally. Dependence of the secondary protein partner (MSH6 or PMS2) on the primary protein partner (MSH2 or MLH1) for stability results in characteristic immunohistochemical patterns, where negative nuclear staining results for both MSH2 and MSH6 suggest an *MSH2* alteration, whereas the lack MLH1 and PMS2 proteins indicates an *MLH1* change. The absence of MSH6 alone points to defective *MSH6* and isolated loss of PMS2 to an altered *PMS2* gene. However, exceptions to these basic rules exist (for example, patients with isolated loss of MSH6 may show a predisposing variant in *MSH2*[62,118]), suggesting that constitutional testing should not be restricted to only the gene predicted from the immunohistochemical pattern but should cover other relevant alternatives as well.

In patients with CRC and absent MLH1 and PMS2, tumor testing for *MLH1* promoter methylation and/or *BRAF* V600E is useful to rule out likely sporadic cases before proceeding to constitutional testing. Detection of a somatic *BRAF* V600E change is closely associated with *MLH1* hypermethylation, which accounts for approximately 70% of consecutive MMR-deficient CRCs.[60,61] The presence of *BRAF* V600E and methylation of *MLH1* promoter (affecting region C specifically[119]) in colon cancer strongly argue against LS.[91,120] This prediction is not without exceptions. In a literature review by Parsons et al,[120] 4 of 550 CRCs (1.4%) from known MMR gene variant carriers and in another large cohort,[121] 15 of 969 (1.6%) of LS CRCs showed *BRAF* V600E. Moreover, somatic *MLH1* promoter methylation may, although rarely, accompany a pathogenic *MLH1* constitutional variant as a "second hit."[122] In EC, oncogenic *BRAF* variants are rare,[123] and it is not possible to use *BRAF* V600E as a proxy for somatic *MLH1* hypermethylation in that (or other noncolorectal) context.

Next-generation tumor sequencing may provide an alternative approach to the universal screening method.[124] Tumor sequencing alone had better sensitivity than MMR protein expression analysis plus *BRAF* and MSI plus *BRAF* and equal specificity to MMR protein expression analysis plus *BRAF* and MSI plus *BRAF*. Constitutional sequencing of the respective MMR gene(s) needs to confirm the results from next-generation tumor sequencing. In addition to heritable changes of MMR genes, tumor sequencing followed by constitutional analysis could detect double somatic MMR gene alterations responsible for LLS (Table 2). Finally, tumor sequencing could reveal actionable therapeutic targets, such as *KRAS*, *NRAS*, or *BRAF* mutations, that could inform chemotherapy choice.[124]

In patients with suspected LS but no tumor sample available for analysis, clinical prediction models such as PREMM (PREdiction Model for gene Mutations)[125] may be useful when assessing the need for constitutional testing.[109,110] Recently, it has become possible to test MMR in nonneoplastic tissue and, thus, diagnose LS regardless of clinical affection status or family history. Early small-pool polymerase chain reaction analyses were able to detect MSI in peripheral blood leukocytes from LS patients. However, the labor intensiveness of the method and ambiguity of the results at low and high extremes (false negatives in LS patients and false positives in older healthy individuals) prevent applying the method to clinical diagnostics.[126]

Subtle signs of reduced MMR gene function in nontumorous cells[127,128] provide the basis for a novel approach, the DiagMMR test (LS CancerDiag Ltd).[129] The test uses skin fibroblasts. It is commercially available for *MSH2* and *MSH6* and yields clinically actionable results without the need to find or interpret DNA variants.

Constitutional Genetic Testing

Irrespective of the screening method used, the diagnosis of LS ultimately requires direct molecular evidence, typically the demonstration of a (likely) pathogenic constitutional variant in one of the MMR genes. The analysis should cover small sequence changes and large rearrangements (achievable by DNA sequencing plus copy number variant analysis by, eg, multiplex ligation-dependent probe amplification) and, in selected cases, constitutional epimutations as well. Blood-based RNA genetic testing used alongside DNA testing can facilitate the interpretation of sequence alterations, including aberrant splicing[130,131] as well as genomic duplications[132] and other large rearrangements.

Recently, multigene panel sequencing has become an alternative to traditional, syndrome-specific gene tests. By multigene testing, 9.0% of patients suspected of having LS showed (likely) pathogenic variants of MMR genes or *EPCAM*, whereas 5.6% of the patients had variants in other cancer predisposition genes.[133] LS accounted for 3% and other cancer predisposition genes for 7% of consecutive patients with CRC[8] or EC[134] unselected for high-risk features. A retrospective review of patients undergoing multigene panel testing without preselection for clinical or family features of LS showed that 27.5% of MMR gene variant carriers presented with hereditary breast and ovarian cancer phenotype.[135] Current LS screening guidelines might miss many of those cases. In a recent prospective investigation, universal multigene panel testing in CRC resulted in improved detection of pathogenic variants (affecting MMR genes in 3.1% and BRCAness-related genes in 6.4% of the patients) over guideline-based testing. Genetic findings modified clinical management in 1 in 10 patients.[136] Current National Comprehensive Cancer Network guidelines[137] suggest consideration of multigene testing for all new CRC patients. Although the possibility to identify cancer susceptibility beyond LS is an advantage of multigene panel testing, a frequent occurrence of variants of uncertain significance of no immediate clinical utility[8,133] is a potential drawback unless additional methods are available for follow-up.

Implications of Molecular Advances in Cancer Surveillance, Prevention, and Treatment

Surveillance Recommendations

Increased understanding of the gene-associated differences in risk has turned clinical management guidelines gene specific (Table 3). This has resulted in de-escalation of some of the previous recommendations, especially for *MSH6* and *PMS2* carriers. For example, because *PMS2* heterozygous carriers present with a low cumulative incidence of CRC and likely have good efficacy of colonoscopy surveillance because the MMR defect is not the primary driver of CRC, colonoscopy recommendations in Europe have been relaxed.[138,139] Current European[138,139] and US[137,140] recommendations state that colonoscopy surveillance may start later in *MSH6* and *PMS2* variant carriers. Similarly, association of urothelial cancer with *MSH2* variants is making urologic screening recommendations more gene specific.[141]

It is unknown if gastric/upper gastrointestinal carcinogenesis in LS is a stepwise and anticipated or fast and unpredictable process. Moreover, the role that *Helicobacter pylori* may play in it remains unsettled.[142] Incomplete understanding of the pathogenesis has led to considerable variability in surveillance recommendations.[143] Future studies will indicate which preventive arm would be the most efficient—an endoscopic, chemoprevention, or lifestyle-based approach.

Lifestyle and Aspirin Chemoprevention

Increased physical activity and reduced body adiposity are associated with decreased cancer risk in the general population, and the same applies to LS.[144,145] A recent study[146] showed the impact of high-intensity physical activity and weight maintenance on CRC prevention, especially in male LS patients. Weight status or weight change may not be associated with EC risk in LS.[147] In the aspirin chemoprevention trial Concerted Action Polyposis Prevention 2, overweight increased CRC risk in a gene-specific manner, being significant in *MLH1* but not *MSH2* variant carriers.[148] Thus, sex and the predisposing MMR gene may modify the impact of lifestyle factors on cancer risk in LS. Importantly, aspirin may abrogate the increased cancer risk related to obesity.

Treatment of Lynch Syndrome Cancers

Available evidence supports the adjustment of surgical management of CRC according to the predisposing MMR gene.[138] *MLH1* and *MSH2* variant carriers have a high risk of metachronous CRC,[92] and extended surgery would decrease the need for further bowel surgery. However, extended resection does not directly improve CRC-related mortality.[149] Lower cumulative risks of primary and metachronous CRC in *MSH6* and *PMS2* variant carriers[92] would justify standard segment resection. However, a history of painful and cumbersome surveillance colonoscopies would support extended bowel resection also in *MSH6* and *PMS2* patients because postoperative bowel adhesions may further complicate the endoscopy.

Verified lymph node metastases or other high-risk factors (like blood vessel invasion) may indicate postoperative adjuvant therapy after curative resection of CRC. The main principles adopted from sporadic CRC apply to LS patients as well. However, in certain respects, LS and other MSI-H/dMMR CRC patients constitute a distinct oncologic subgroup. Patients with MSI-H/dMMR CRC might not benefit

Table 3. NCCN[137,140] and European[111,138,139,141] Guidelines for Cancer Surveillance in LS

	NCCN guidelines				European guidelines			
Organ-specific surveillance	*MLH1*	*MSH2*	*MSH6*	*PMS2*	*MLH1*	*MSH2*	*MSH6*	*PMS2*
Colorectal surveillance								
Age at initiation, *y*	20–25	20–25	30–35	30–35	25	25	35	35
Screening interval, *y*	1–2	1–2	1–3	1–3	2–3	2–3	2–3	3–5
Gastroduodenal surveillance								
Age at initiation, *y*	40 with risk factors	40 with risk factors	40 with risk factors	40 with risk factors	No recommendation	No recommendation	No recommendation	No recommendation
Screening interval, *y*	3–5	3–5	3–5	3–5	No recommendation	No recommendation	No recommendation	No recommendation
Gynecologic surveillance								
Age at initiation, *y*	30–35	30–35	30–35	30–35	25	25	25	25
Screening interval, *y*	1–2	1–2	1–2	1–2	Optional annual review	Optional annual review	Optional annual review	Optional annual review
Urologic surveillance								
Age of initiation, *y*	30–35	30–35	30–35	30–35	45–50	45–50	45–50	45–50
Screening interval, *y*	No recommendation	No recommendation	No recommendation	No recommendation	Urinalysis and urine cytology, abdominal ultrasound every 2 years	Urinalysis and urine cytology, abdominal ultrasound yearly	Urinalysis and urine cytology, abdominal ultrasound every 2 years	Urinalysis and urine cytology, abdominal ultrasound every 2 years
Colon cancer surgery	Colectomy with ileorectal anastomosis	Colectomy with ileorectal anastomosis	Colectomy with ileorectal anastomosis	Colectomy with ileorectal anastomosis	Colectomy with ileorectal or ileosigmoid anastomosis	Colectomy with ileorectal or ileosigmoid anastomosis	Standard resection	Standard resection
Risk-reducing gynecologic surgery, *y*	Individualized	Individualized	Individualized	Individualized	35–40	35–40	35–40	35–40
Use of aspirin in CRC chemo-prevention, *mg/day*	Individualized	Individualized	Individualized	Individualized	>75–100	>75–100	>75–100	>75–100

NCCN, National Comprehensive Cancer Network.

from the traditionally used 5-fluorouracil–based regimen,[150] although there is evidence to support the use of FOLFOX (FOLinic acid, Fluorouracil, OXaliplatin) in CRC patients with stage 3 disease irrespective of MMR status.[151] Recent results from adjuvant or neoadjuvant immunotherapy are especially encouraging. Checkpoint inhibitors have displayed promising responses compared to traditional regimens in metastatic dMMR CRCs[152] and may be used as first-line therapy.[153–155] PD-1 blockade with dostarlimab alone showed remarkable rates of complete clinical response in locally advanced MSI-H/dMMR rectal cancer, truly enabling organ-sparing approaches in the future.[85] LS and sporadic MSI cancer patients appear to respond similarly.[156]

Vaccine-Based Immunotherapy and Immunoprevention

Vaccination with immunogenic frameshift peptides may offer a promising future approach for the treatment and prevention of LS-associated cancers. A phase 1 study with a Nous-209 tested an off-the-shelf polyvalent vaccine targeting 209 shared frameshift peptides in 12 patients with metastatic MSI-H CRC, gastric, or gastroesophageal junction cancers combined with immunotherapy with pembrolizumab. Seven patients had partial response, 2 had stable disease, and 3 had progressive disease, with no dose0limiting toxicity.[157] Another phase 1/2a clinical trial included 4 vaccinations of 3 frameshift peptide neoantigens over 6 months. All 16 patients with a previous dMMR CRC showed humoral and cellular immune responses and no adverse effects.[158] A phase 1b/2 trial on Nous-209 as a single agent will recruit 45 LS carriers with primary endpoints of safety, tolerability, and immunogenicity (NCT05078866). So far, the cancer-preventing efficacy of frameshift peptide vaccines derives from a murine model showing reduced tumor growth and improved survival in *MSH2*-deficient mice.[159]

Prospective Vision

Along with new modalities for cancer prevention, efficient and early detection of LS predisposition becomes increasingly important. The current tumor testing and constitutional sequencing-based variant detection needs to be supplemented with other approaches, such as methods capable of diagnosing heritable predisposition even in cancer-free individuals and techniques that, by monitoring function rather than sequence, can aid in the interpretation of variants of uncertain significance and cases with clinical suspicion of LS but no identifiable sequence changes. DiagMMR (discussed earlier) is one such method. Moreover, dMMR crypts are a specific biomarker of LS, and immunohistochemical evaluation for such crypts in colonoscopic biopsy samples of normal mucosa may help identify LS patients.[160]

Frequent CRCs despite regular colonoscopic surveillance[74] and late-onset cancers (of the upper gastrointestinal tract, eg, that may be associated with poor prognosis) in LS individuals who survived their first and second cancers[93] set new requirements for cancer surveillance. At the same time, the combined epidemiologic and molecular evidence is expanding the spectrum of organs that may be cancer prone in genetically predisposed individuals. Liquid biopsies could constitute a minimally invasive and sensitive approach for LS cancer screening[161] and warrant further research. Selection of LS patients for adjuvant therapy is another possible area where liquid biopsies might prove valuable. The high cumulative cancer risk in LS carriers and the expanding LS tumor spectrum modify the requirements for cancer prevention as well. According to recent findings,[162] resistant starch may prevent LS extracolonic cancers, including those of the upper gastrointestinal tract that are difficult to detect and manage. Finally, leveraging the unique immunologic characteristics of LS[88] to multiorgan cancer prevention may offer an efficient new tool because these biological processes are operative in histologically normal tissues already and apply even to LS individuals who have never been diagnosed with cancer.

Because clinical presentation may significantly vary even in individuals with identical genotypes, it is obvious that additional genetic and nongenetic factors contribute to clinical phenotype. Identification of cancer-protective factors is a particularly interesting area of future research. Observations of some 8% of carriers of pathogenic MMR gene variants never developing cancer during their lifetime[163] suggest that such factors do exist. International consortia of LS investigators are ideal to be in charge of investigations on phenotype modifiers to guarantee sufficiently large study series with worldwide coverage.

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Conflicts of interest
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