Molecular Mechanism of Lynch Syndrome

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Abstract

Lynch syndrome is a cancer-predisposing syndrome inherited in an autosomal dominant manner, wherein colon cancer and endometrial cancer develop frequently in the family, it results from a loss of function of one of four different protein (MLH1, MSH2, MSH6, and PMS2), which are the products of mismatch repair genes. An abnormal *EPCAM* gene at the position adjacent to the *MSH2* gene also inhibits *MSH2* expression and causes Lynch syndrome.

Mismatch repair proteins are involved in repairing of incorrect pairing, including point mutations and deletion/insertion of simple repetitive sequences, so-called microsatellites, that can arise during DNA replication. MSH2 forms heterodimers with MSH6 or MSH3 (MutSα, MutSβ, respectively) and is involved in mismatch-pair recognition and initiation of repair. MLH1 forms a complex with PMS2 and functions as an endonuclease. If the mismatch repair system is thoroughly working, genome integrity is maintained at a high level. Lynch syndrome is a state of mismatch repair deficiency (MMRd) due to a monoallelic abnormality of the mismatch repair genes. The phenotype indicating the mismatch repair deficiency can be frequently observed as a microsatellite instability (MSI) in tumors.

Generally, Lynch syndrome develops in adulthood, but MMR gene abnormalities are observed in children with different genotypes and phenotypes. Children with germline biallelic mismatch repair gene abnormalities were reported to develop conditions such as gastrointestinal polyposis, colorectal cancer, brain cancer, leukemia, and so on. This condition is called constitutional mismatch repair deficiency (CMMRD).

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In addition, for promoting cancer genome medicine in a new era, such as by utilizing immune checkpoints, it is important to understand the genetic and genomic molecular background, including the status of mismatch repair deficiency.

Keywords

Mismatch repair gene · Lynch syndrome · Microsatellite instability · Constitutional mismatch repair deficiency · Immune checkpoint inhibitor

Abbreviation

CMMR-D	Constitutional mismatch repair deficiency
CNS	Central nervous system
CTE	Congenital tufting enteropathy
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
IHC	Immunohistochemical staining
ISI	Immune checkpoint inhibitor
MLPA	Multiple ligation-dependent probe amplification
MMR	Mismatch repair
MSI	Microsatellite instability
PCNA	Proliferating cellular nuclear antigen
PD-1	Programmed cell death protein 1
RFC	Replication factor
TMB	Tumor mutational burden

1.1 Introduction

Cancer is fundamentally a genetic disease, and pathogenic variants, also called "mutation," are pivotal to its etiology and progression. Carcinogenesis develops by the accumulation of numerous genetic and epigenetic abnormalities [1–4]. Therefore, cancer has the following six characteristics: sustained proliferative signaling, evasion of growth suppressors, resistance cell death, replicative immortality, angiogenesis induction, and activation of invasion and metastasis [5]. Therefore, elucidation of carcinogenesis is essential for therapeutic development [6]. Although rare, hereditary cancer syndromes are observed in cancers derived from any organ. In individuals with hereditary cancer syndrome, the initial cancer-causing pathogenic variant is inherited through the germ cell and therefore, is already present in all 37 trillion cells that make up the body. Lynch syndrome (MIM# 120435) is an autosomal dominant syndrome with a penetration rate of about 80% characterized by several individuals in the family affected with colorectal cancer (CRC) or extracolonic tumors of the endometrium, stomach, small bowel, ureter, renal pelvis, ovary, and hepatobiliary tract [7].

Lynch syndrome occurs due to loss of function of the mismatch repair mechanism for genomic replication errors. This article outlines the basis of molecular genetics involved in Lynch syndrome.

1.2 DNA Repair System

The frequency of replication errors is 10^{-10} per base of DNA per cell division, and in an estimated 10^{15} cell divisions during an individual's lifetime replication errors cause thousands of new DNA variants in the genome in every cell. Eukaryotes have multiple repair systems to avoid replication errors (Table 1.1) [8]. Maintaining DNA-integrity through genome repair suppresses cancer development and progression by genomic abnormalities. Genes encoding molecules involved in genome repair are referred to as DNA repair genes and "caretaker tumor suppressor genes."

Table 1.1 DNA repair systems and predisposition to cancer [8]

DNA repair	Damage	Characteristics	Predisposition
Base excision Repair (BER)	Single strand	Repair mechanism for a single nucleotide in a single strand of DNA that is generated through oxidation (e.g., 8-oxoguanine), alkylation (e.g., methylation), and deamination. No ATP required	*MUTYH*-associated polyposis (MAP)
Nucleotide excision repair (NER)	Single strand	Repair mechanism against damage that causes DNA structure change over several tens of base pairs via pyrimidine dimer formation by ultraviolet exposure. ATP required	Xeroderma pigmentosum Cockayne Syndrome
Mismatch repair: (MMR)	Single strand	Repair mechanism of base mismatch pairing caused in DNA replication (S phase). Usually, it corresponds to an error of one to several base pairs ATP required	Lynch syndrome
Proofreading repair	Single strand	It occurs done during DNA replication. In E. coli, 3′→5′ exonuclease of DNA polymerase I has this function. In humans, involvement of enzymes other than DNA polymerase is also conceivable	Polymerase proofreading associated polyposis (PPAP)
Homologous recombination (HR)	Double strand	When double stranded breaks occur in S phase/G2 phase, the cleaved portion of a normal allele is used as the template DNA. This mechanism restores the original sequence by recombination	Hereditary breast and ovarian cancer (HBOC)
Nonhomologous end-Joining (NHEJ)	Double strand	In double strand breaks in the G1 phase, this repair mechanism concentrates multiple molecules on the excised ends and directly combines them. In this repair, some nucleotides around the break part may be missing in some cases	LIG4 syndrome

Table 1.2 DNA repair system for replication errors in *Escherichia coli* [8]

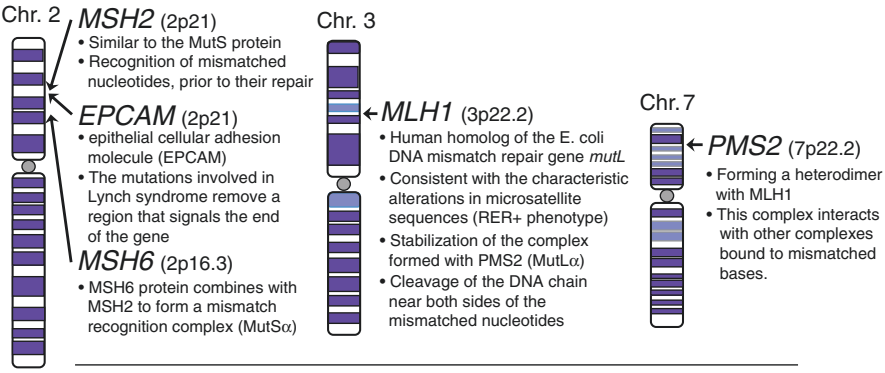
Step	Pathway	Protein activities	Mutation rate $\begin{pmatrix}\text{per nucleotide}\\ \text{per generation}\end{pmatrix}$
1	DNA synthesis	5′→3′-elongation activity of DNA polymerase III(α) (1000 nucleotides/s.)	10^{-5}–10^{-6}
2	Proofreading	3′→5′-exonuclease activity of DNA polymerase III(ε)	10^{-7}
3	Mismatch correction	Mismatch correction proteins Mut S, Mut L, Mut H etc.	10^{-9}–10^{-10}

The mismatch repair system was recognized in 1961, with proposal that the correction of DNA base pair mismatches within recombination intermediates is the basis for gene conversion [9]. Elucidation of the mismatch repair system has been advanced by fundamental research based on *Escherichia coli*, developed four *E. coli* mutator genes: *mutH*, *mutL*, *mutS*, and *uvrD* [10–13]. Inactivation of any of these genes increases the generation of variants in the *E. coli* cell by 50-to 100-fold, indicating the importance of this pathway in variant avoidance and genetic stability. The reduction in mutability afforded by the *E. coli* methyl-directed system has been attributed to its role in the strand-specific elimination of DNA errors (Table 1.2) [6, 8, 14–19]. Research on the mismatch repair system has advanced extensively and has clarified its mechanism and role as an essential mechanism for maintaining genome integrity in organisms and involved in predisposition to cancer development.

1.3 Genes Responsible for Lynch Syndrome

Lynch syndrome, is called hereditary nonpolyposis colorectal cancer: HNPCC in the past, is an autosomal dominant inherited disorder caused by germline pathogenic variants in DNA mismatch repair (MMR) genes. Patients with Lynch syndrome are at an increased risk of developing tumors from a young age and throughout their lifetime. Most of them suffer from multiple synchronous and/or metachronous primary tumors. Colorectal cancer and endometrial cancer (female) are well known in the tumor spectrum of Lynch syndrome. In addition, patients with Lynch syndrome have high potential for developing cancer of the urinary tract, the stomach, the small intestine, the biliary tract, the skin, the brain, and others.

Multiple types of human mismatch repair (MMR) proteins have been discovered and several encoding genes have been isolated so far. Currently, four types of MMR genes, *MLH1* (MIM# 120436), *MSH2* (MIM# 609309), *MSH6* (MIM# 600678), and *PMS2* (MIM# 600259), are used in the clinical applications related to Lynch syndrome. An outline of the responsible genes is shown in Fig. 1.1. The *EPCAM*, which encodes a cell adhesion molecule, is not an MMR gene, but its structural abnormality causes Lynch syndrome, because it is adjacent to the *MSH2* gene [20]. This content will be described later.



Gene	MIM	Locus	No.of exon	CDS (nt)	Product no. of amino acid	MW (kDa) of protein
MLH1	*120436	3p22.2	19	2218	756	84.6
MSH2	*609309	2p21	16	2802	934	104.7
MSH6	*600678	2p16.3	10	4080	1360	152.8
PMS2	*600259	7p22.1	15	2586	862	95.8
EPCAM	*185535	2p21	9	942	314	35

Fig. 1.1 The genes responsible for Lynch syndrome [8]

In 1993, *MSH2* gene was isolated at chromosome 2p22–p21 in 1993 and has high homology with the mutator phenotype gene, mutS of *E. coli* [21–24]. In 1994, as the second responsible gene of Lynch syndrome, *MLH1*, the *E. coli mutL* homologue, was isolated from 3p22.2 [25–27]. In 1995, mismatch binding factors were found as the 100 kD MSH2 or as heterodimers of the 160 kD polypeptide called GTBP/MSH6 (for G/T binding protein), which was recognized as a new member of the MutS homologue [28, 29]. *MSH6* gene was first reported by Japanese researchers as a gene responsible for Lynch syndrome [30, 31]. In 1994, a germline deletion of the *PMS2* was also identified in families with Lynch syndrome. Moreover, additional deletions in tumor samples with microsatellite instability high (MSI-high) showed the presence of two-hits [32, 33].

1.4 Structure and Function of MMR Proteins

Each MMR protein encoded by the corresponding MMR gene has a unique function in repairing replication errors. Therefore, MMR proteins possess unique functional domains. When pathogenic variants of MMR genes occur in the DNA site corresponding to the functional domain, DNA repair function may be impaired. Schematic representations of MLH1, MSH2, MSH6, and PMS2 proteins are shown in Fig. 1.2 [8, 34–38]. Both MLH1 and PMS2 have an ATP binding domain and require ATP molecules for the endonuclease function.

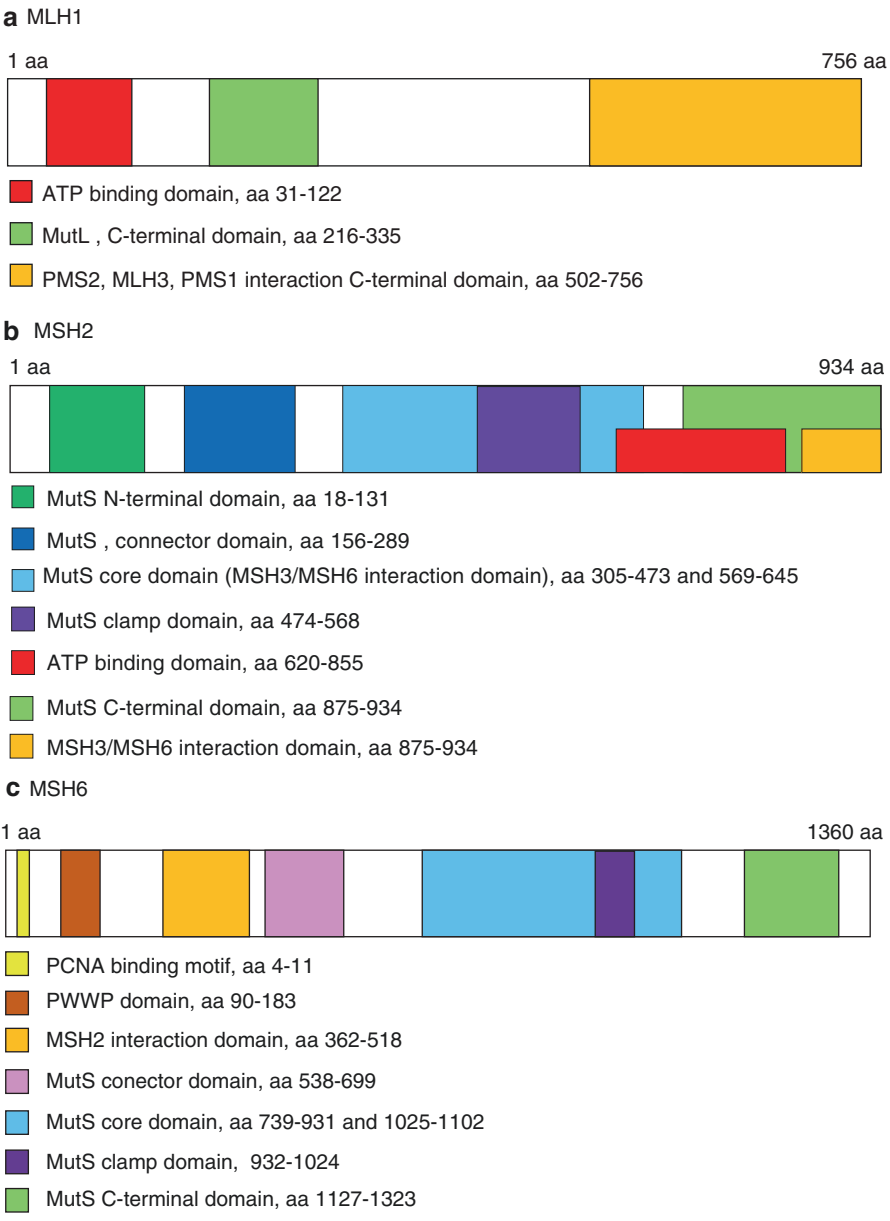


Fig. 1.2 Structure of mismatch repair proteins (**a**) *MLH1*, (**b**) *MSH2*, (**c**) *MSH6*, (**d**) *PMS2* [8]

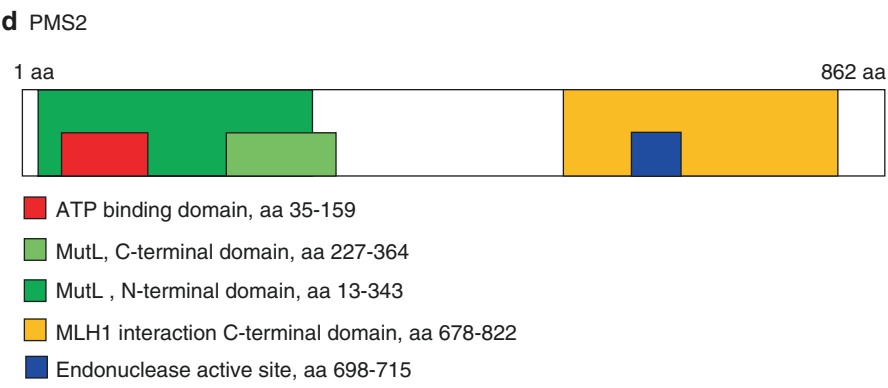


Fig. 1.2 (continued)

Many human MMR related proteins have been identified as homologues of *E. coli* MMR proteins [8, 22–29, 39–49]. These include human homologues of MutS, MutL, ExoI, DNA polymerase δ (pol δ), proliferating cellular nuclear antigen (PCNA), replication factor (RFC), DNA ligase I, and so on. MSH2 heterodimerizes with MSH6 or MSH3 to form MutSα or MutSβ, respectively. These are involved in the mismatch-pair recognition and initiation of repair [50–54]. In addition, various kinds of complexes such as MutLα, MutLβ, and MutLγ are formed and involved in the mismatch repair system [37, 38, 40, 51, 52, 54–63].

1.5 Mechanisms of Mismatch Repair

The mismatch repair (MMR) system consists of sequential steps for the recognition, removal, and resynthesis of the mismatch site in DNA. This system that maintains DNA fidelity is well conserved from *E. coli* to eukaryotes. A schematic diagram of the pathway is shown in Fig. 1.3 [8, 53, 58, 60, 62–82]. Base–base mismatches in double-strand DNA are recognized by MutSα (heterodimer of MSH2-MSH6). MutSα binds as a sliding clamp around the double-strand DNA. MutSα and MutLα form a tetrameric complex and then initiate the process of mismatch repair. The tetrameric complex recruits proliferating cell nuclear antigen (PCNA), replication factor C (RFC), exonuclease 1 (Exo 1) to remove the nascent (daughter) strand, and resynthesize the correct strand. Then, exonuclease 1 (Exo 1) is recruited and removes the nascent (daughter) strand around the error region. The resynthesis step is accomplished by DNA polymerase (Polδ or Polε) and Ligase 1.

1.6 Relationship Between MMR System and DNA Damages

Depending on the DNA damage pattern, specific mismatch repair molecules, and complexes are involved. The outline is shown in Fig. 1.4 [8, 50, 64, 66, 83–86]. The MutSα (heterodimer of MSH2-MSH6) contributes to mismatch recognition by

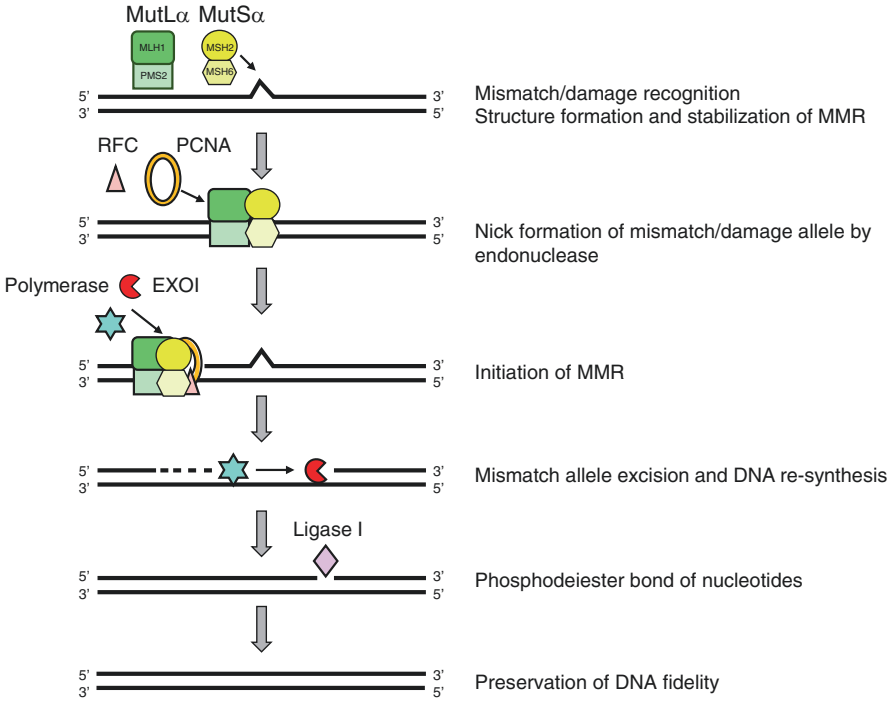


Fig. 1.3 Mechanistic model of mismatch repair [8]

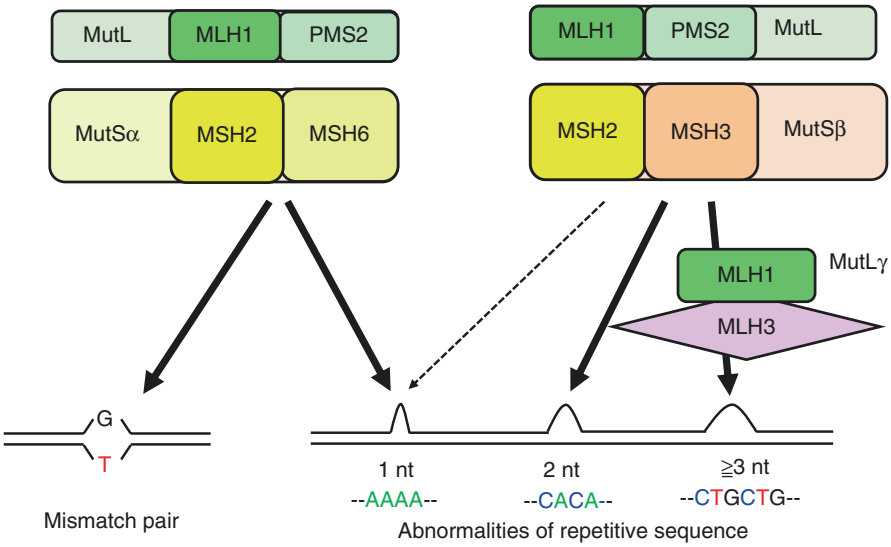


Fig. 1.4 Schematic of DNA damage recognized by the mismatch repair pathway [8]

single nucleotide substitution (e.g., G:T mismatch pair) and recognition of small insertion-deletion loops (IDL, e.g., error of the repeat number in adenine clusters), whereas MutSβ (heterodimer of MSH2-MSH3) contributes to the repair of small loops and relatively large damages up to about ten nucleotide loops. Recently, the function of MutSβ has attracted attention for its biological characteristics and as a prognostic factor of elevated microsatellite instability at selected tetranucleotide (EMAST) colorectal cancer, which shows instability in the repeat sequence of the tetranucleotides [87–91].

1.7 *EPCAM* as the Gene Responsible for Lynch Syndrome

EPCAM is located at 2p21 adjacent to the *MSH2* on the 5′ upstream and encodes the EPCAM protein, expressed on the membrane of cells in epithelial tissues and plasma cells, and is involved in cell-cell adhesion function [92, 93]. Although *EPCAM* is not the direct responsible gene of Lynch syndrome, but it is located just 17 kb upstream of *MSH2*. The deletion of *EPCAM* affects *MSH2* gene expression, resulting in Lynch syndrome. The schema is shown in Fig. 1.5 [8, 20]. The *cis*-deleted alleles inhibit *MSH2* expression and finally causes Lynch syndrome in 1.3% of the affected families [20, 94].

In addition, biallelic inactivation of *EPCAM* is responsible for congenital tufting enteropathy (CTE, MIM# 613217) with an estimated incidence of one in 50,000–100,000 births in Western Europe [95–98]. CTE presents within the first months of life with severe chronic watery diarrhea and growth restriction. *EPCAM* abnormalities responsible for CTE are usually missense mutations, nonsense mutations, minute insertions/deletions, and splicing errors, unlike Lynch syndrome [98].

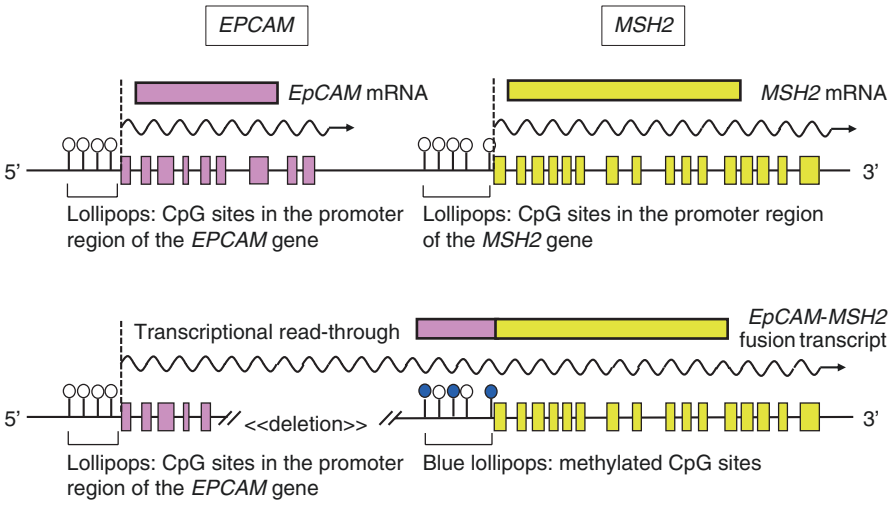


Fig. 1.5 A *cis*-deletion of *EPCAM* gene causes an epimutation of the *MSH2* gene [8]

1.8 Constitutional Mismatch Repair Deficiency Syndrome

Constitutional mismatch repair deficiency syndrome (CMMR-D) is caused by biallelic homozygous or compound heterozygous pathogenic germline pathogenic variants of MMR genes and is a distinct childhood cancer preposition syndrome (MIM# 276300) with an autosomal recessive inheritance [99–101]. In biallelic germline pathogenic variant carriers of MMR genes, hematological malignancies, brain/central nervous system (CNS) tumors, and Lynch syndrome associated carcinomas develop frequently. In the gastrointestinal tract, bowel adenomatous polyposes are often observed as premalignant lesions that require differential diagnosis from FAP. By the way, the pathological condition classified as a subtype of FAP called Turcot's syndrome is considered to be exactly CMRR-D [102, 103].

The median age at diagnosis of hematological malignancies and brain/CNS tumors was respectively, 6.6 (age range: 1.2–30.8) and 10.3 (age range: 3.3–40) years. However, Lynch syndrome-associated tumors developed later (median age at diagnosis: 21.4 years (age range: 11.4–36.6)). Moreover, the spectrum of Lynch syndrome is mostly colorectal cancer and/or endometrial cancer [104]. Various non-neoplastic features are related to CMMR-D including Cafe au lait spots (NF1 like), skin hypopigmentation, mild defects in immunoglobulin class switching recombination, agenesis of the corpus callosum, cavernous brain hemangioma, capillary hemangioma of the skin, combination of various congenital malformations, and lupus erythematosus.

1.9 Genetic Testing for Lynch Syndrome

In order to select high-risk individuals with Lynch syndrome from among patients with colorectal cancer and to increase the efficiency of detecting germline pathogenic variants, microsatellite instability (MSI) testing and/or immunohistochemical staining (IHC) of MMR proteins is recommended as universal tumor screening and is recommended to do first [102, 105, 106]. The MSI testing is a method to easily identify events in which genetic integrity has been damaged due to repair failures of DNA replication errors using simple repeated microsatellite sequences [107–111]. Five types of repeat markers including mononucleotide and dinucleotide repeats have been used, but recently mononucleotide repeat markers have been preferred. Cases with different numbers of repeats between normal tissue-derived DNA and cancer-derived DNA are considered as positive [112]. If two or more of the five markers show instability, the tumor is evaluated as MSI-high (MSI-H). The results of MSI-H colorectal cancer are shown in Fig. 1.6. If one of the markers shows instability, the tumor is considered as MSI-low (MSI-L). If positive markers are not observed, the mismatch repair system is evaluated to be proficient and is called MS-stable (MSS).

Immunohistochemical staining of MMR proteins can reveal damaged molecules using specific antibodies. Staining with four antibodies: MLH1, MSH2, MSH6, and PMS2 can predict the gene causing Lynch syndrome (Table 1.3) [113–120].

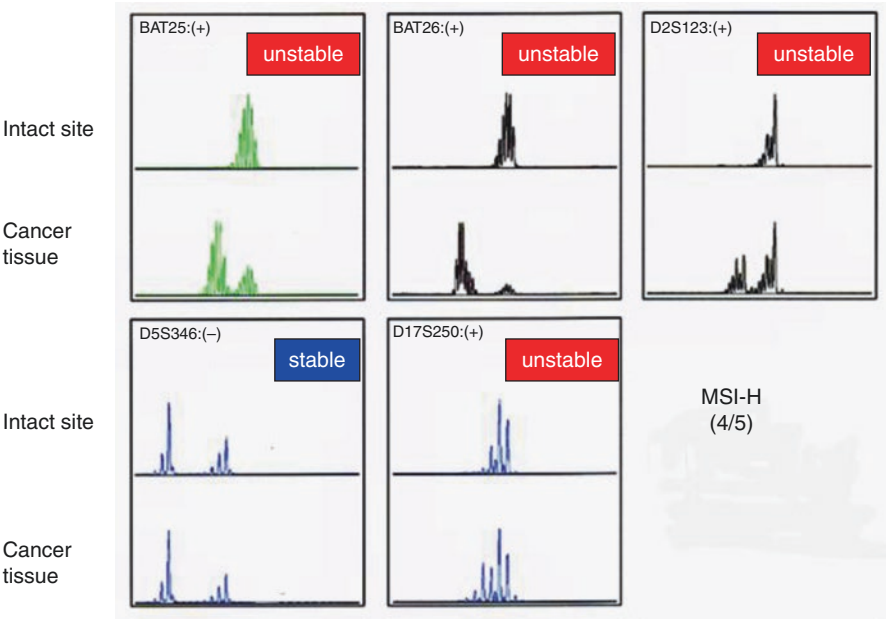


Fig. 1.6 Analytic image of MSI testing: four out of five markers show microsatellite instability [8]

Table 1.3 IHC findings associated with *MLH1*, *MSH2*, *MSH6*, and *PMS2* mutations [8]

	IHC staining			
Mutation of MMR genes	MLH1	MSH2	MSH6	PMS2
MLH1	–	+	+	–
MSH2	+	–	–	+
MSH6	+	+	–	+
PMS2	+	+	+	–

For MSI testing, sensitivity ranged from 66.7 to 100.0% and specificity ranged from 61.1 to 92.5%, whereas for IHC staining, sensitivity ranged from 80.8 to 100.0%, and specificity ranged from 80.5 to 91.9% [121].

Approximately 10–15% of sporadic colorectal cancers show MSI-H findings. The cause is mostly the loss of MSH1 protein due to methylation of the *MLH1* gene promoter region. About half of MSI-H sporadic colorectal cancers show *BRAF* V600E mutation, which is rarely detected in colorectal cancers from patients with Lynch syndrome. *MLH1* methylation analysis and *BRAF* V600E mutation testing in colorectal cancers can improve the efficiency of the diagnosis for Lynch syndrome [36, 122].

Final genetic testing for Lynch syndrome is performed using DNA sequencing in selected cases excluding sporadic colon cancer from all colorectal cancers. For a long time, genetic testing has mainly been performed using Sanger sequencing, and multiplex ligation-dependent probe amplification (MLPA) has been adopted

for a wide range of abnormalities such as large deletions/insertions [123]. Clinical genetics is currently transitioning from phenotype-directed single gene testing to multigene panels [124]. Multigene panel testing using next generation sequencing for hereditary colorectal cancer has been evaluated as a feasible, timely, and cost-effective approach compared to single gene testing [125]. Previously, the distribution of germline pathogenic variants in MMR and *EPCAM* genes in Lynch syndrome was thought to predominantly occur in *MSH2* and *MLH1* and less frequently in *MSH6* and *PMS2*. As a result of multigene panel testing without universal tumor screening, Espenschied et al. reported that *MSH6* pathogenic variants were the most frequent, followed by *PMS2*, *MSH2*, *MLH1,* and *EPCAM* (Table 1.4) [8, 123, 126–128]. About 12% of individuals carrying MMR gene pathogenic variants have breast cancer alone. Furthermore, even MMR gene pathogenic variant carriers do not always meet the criteria for Lynch syndrome or the *BRCA1*/*BRCA2* testing criteria. However, *MSH6* and *PMS2* germline pathogenic variants are associated with an increased risk for breast cancer [126, 129]. Table 1.4 shows the gene-specific distributions of germline variants by the types of abnormalities in mismatch repair genes. Most *MSH2, MLH1,* and *MSH6* pathogenic variants were truncated types such as nonsense mutations or frameshift mutations [8, 130]. Knowledge of choice of analysis method is important. A wide range of rearrangements were detected at 10%, 7%, and 10% for MSH2, MLH1, and PMS2, respectively. Therefore, the selection of an appropriate analysis method is required for genetic testing.

Table 1.4 Germline mutation analyses in the responsible genes in Lynch syndrome [8]

(a) Distribution of mutations in overall mismatch repair genes and *EPCAM*

Gene	*MSH2* (%)	*MLH1* (%)	*MSH6* (%)	*PMS2* (%)	*EPCAM* (%)	Publication year	Ref#
Distribution of mutations in overall mismatch repair genes and *EPCAM* gene	23.7	21.6	29.4	24.2	1.2	2017	[126]
	21.2	39.4	18.2	21.2	–	2017	[127]
	36	40	18	6	–	2016	[128]
	34	40	18	8	–	2014	[129]

(b) Distribution of the types of germline variants in mismatch repair genes [130]

Variant type	*MSH2* (%)	*MLH1* (%)	*MSH6* (%)	*PMS2* (%)
Missense	31	40	49	62
Nonsense or frameshift	49	40	43	24
In-frame	2	2	3	1
Splice	8	11	3	3
Large rearrangement	10	7	2	10

1.10 Effectiveness of Immune Check Point Blockades and a Hypermutable State (High Tumor Mutational Burden)

As cancer cells escape the host immune system by suppressing T cell activation, thus exert an immunosuppressive function due to immune checkpoint molecules. The immune checkpoint molecules include cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1, CD279), and so on [131, 132], which were found to negatively control the immune system [133, 134]. In human cancer treatment, anti-PD-1 antibody was found to be effective for non-small cell lung cancer, malignant melanoma, and renal cell cancer and was also clinically applicable in safety [135]. The clinical efficacy of PD-1 inhibitor was found to be higher in mismatch repair-defective colorectal and non-colorectal cancers compared to proficient-mismatch repair cancers [136]. According to recent survey results, as shown in Fig.1.7, high tumor mutational burden (TMB) is an excellent biomarker for predicting the efficacy of immune checkpoint inhibitors (ICIs) [137–139], and the group of colorectal cancer patients with the biological characteristics of mismatch repair deficient (MMRd) has a significantly better response to ICIs

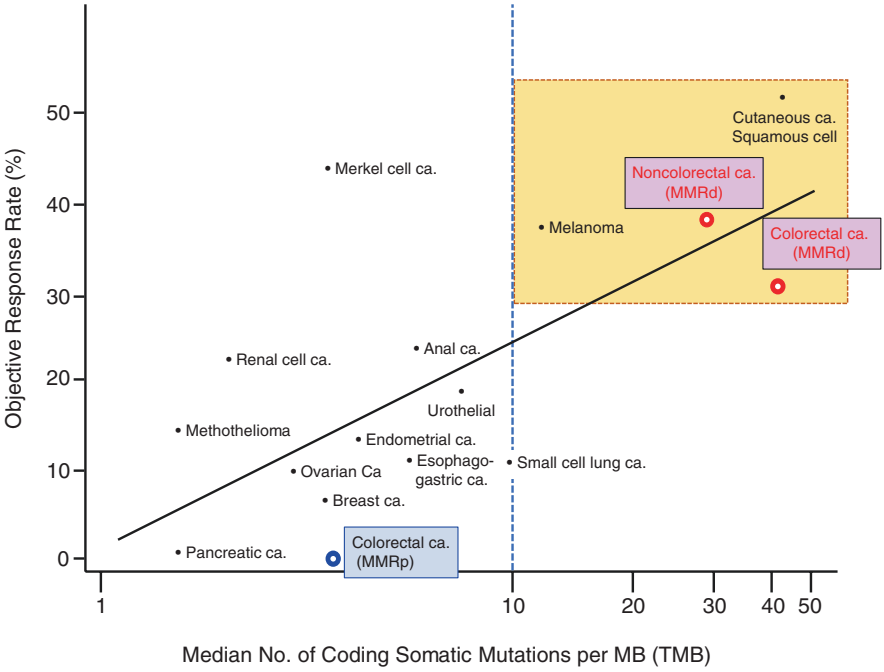


Fig. 1.7 Correlation between tumor mutational burden (TMB) and objective response rate with immune checkpoint inhibitors [137]

than those with mismatch repair proficient (MMRp) [136, 137]. In gastrointestinal cancer, because the state of microsatellite instability high (MSI-H) state has been shown to correlate well with high TMB based on an analysis of many cancer genomes, the microsatellite instability (MSI) testing is used as a standard biomarker to predict the response of ICIs [140–142].

1.11 Future Directions

The cancer-accumulating family reported by Warthin AS more than 100 years ago led to the establishment of Lynch syndrome by the vigorous genetic epidemiological approach of Lynch HT et al. On the other hand, mismatch repair genes have been elucidated as part of the genome integrity system of *Escherichia col*i and yeast. These basic researchers worked together to understand the clinical, genetic, and molecular biological aspects of Lynch syndrome. With its natural history and molecular biological characteristics clarified, presymptomatic diagnosis by genetic testing for at-risk persons in the family, and appropriate medically actionable interventions, such as early diagnosis, are becoming possible.

The development of ICIs is a major milestone in the treatment of patients with Lynch Syndrome. Most malignant tumors in patients with LS have MSI-H status and are expected to respond to ICIs. These studies have shown new possibilities for the treatment of hereditary tumor syndrome. In future, we hope that advances in the integrated understanding of the clinical and molecular biology of Lynch syndrome will lead to the development of novel diagnostic methods and effective treatments.

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Disclosure Statement of COI
The author declares no potential conflicts of interest.
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