Gynecologic Oncology Reports 53 (2024) 101381

Contents lists available at ScienceDirect

Gynecologic Oncology Reports

journal homepage: www.elsevier.com/locate/gynor

Case report

Germline *MLH1* and *MSH6* mutations from two Lynch syndrome families identified in a patient with early-onset of endometrial cancer: A case report

Yi-Ching Huang [a], Peng-Chan Lin [a,b], Pei-Ying Wu [c], Nai-Syuan Chen [d], Meng-Ru Shen [c], Yu-Min Yeh [a,*], Ya-Min Cheng [c,e,*]

[a] Department of Oncology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan
[b] Department of Genomic Medicine, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan
[c] Department of Obstetrics and Gynecology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Taiwan
[d] Department of Pathology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Taiwan
[e] Superintendent, Kuo General Hospital, Tainan, Taiwan

ARTICLE INFO

Keywords:
Lynch syndrome
Endometrial cancer
Colorectal cancer
DNA mismatch repair
MLH1
MSH6

ABSTRACT

Introduction: Lynch syndrome is caused by a germline mutation in mismatch repair (MMR) genes, leading to the loss of expression of MMR heterodimers, either MLH1/PMS2 or MSH2/MSH6, or isolated loss of PMS2 or MSH6. Concurrent loss of both heterodimers is uncommon, and patients carrying pathogenic variants affecting different MMR genes are rare, leading to the lack of cancer screening recommendation for these patients.
Case presentation:
Here, we reported a female with a family history of Lynch syndrome with *MLH1* c.676C > T mutation. She developed endometrial cancer at 37 years old, with loss of MLH1/PMS2 expression. Immunohistochemical staining on tumor samples incidentally detected the additional loss of MSH6 expression. Whole exome sequencing on genomic DNA from peripheral blood revealed *MSH6* c.2731C > T mutation, which was confirmed to be inherited from her mother, who had an early-onset ascending colon cancer without cancer family history.
Conclusion: This is a rare case of the Lynch syndrome harboring germline mutations simultaneously in two different MMR genes inherited from two families with Lynch syndrome. The diagnosis of endometrial cancer at the age less than 40 years is uncommon for Lynch syndrome-related endometrial cancer. This suggests an earlier cancer screening for patients carrying two MMR mutations.

1. Introduction

Lynch syndrome (LS), is an autosomal dominant disorder associated with a spectrum of cancers, including colorectal cancer (CRC), endometrial cancer, and other malignancies (Lynch et al., 2015). The syndrome results from germline mutations primarily in DNA mismatch repair (MMR) genes, such as mutL homolog 1 (*MLH1*), mutS homolog 2 (*MSH2*), mutS homolog 6 (*MSH6*), and PMS1 homolog 2 (*PMS2*) (Lynch et al., 2015). The pathogenesis involves a germline mutation in one of the MMR genes, followed by a second hit in the remaining wild-type allele, resulting in the inactivation of MMR proteins and mismatch repair deficiency (dMMR). This deficiency triggers tumorigenesis as base–base mismatches and insertions/deletions generated during replication remain unrepaired. Tumors with dMMR typically exhibit increased alterations in tandem repeat lengths within microsatellite regions, referred to as microsatellite instability (MSI).

Immunohistochemical (IHC) staining for MMR protein expression and/or MSI testing is currently recommended by guidelines for all endometrial cancer patients to maximize LS screening sensitivity (Network, 2023). Confirmatory germline genetic testing follows to establish the LS diagnosis. Risk for specific cancer types in LS varies with specific MMR gene mutations (Bonadona et al., 2011; Møller et al., 2017). Tailored surveillance and prevention strategies, based on cancer type risk and age of onset, are recommended (Network, 2023). For instance, LS-associated endometrial cancer risk increases significantly after age 40, with suggested screening starting at 30–35 years (Network, 2023; Dominguez-Valentin et al., 2023). However, LS with two MMR gene mutations is rare, requiring more data to establish cancer risk and

* Corresponding authors at: Department of Oncology, National Cheng Kung University Hospital, Tainan 704, Taiwan, No.138, Sheng-Li Road, Tainan, Taiwan, Superintendent, Kuo General Hospital, Tainan, Taiwan, No.22, Sec 2, Minsheng Road, Tainan, Taiwan.
E-mail addresses: i5485111@gmail.com, chengym@mail.ncku.edu.tw (Y.-M. Cheng).

https://doi.org/10.1016/j.gore.2024.101381
Received 14 February 2024; Received in revised form 23 March 2024; Accepted 27 March 2024
Available online 28 March 2024

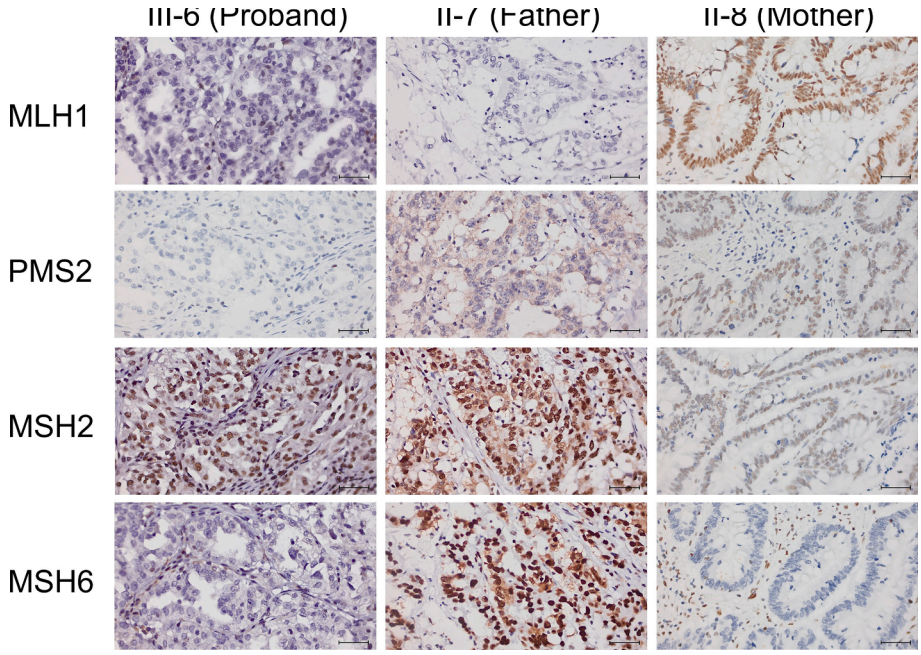

Fig. 1. Immunohistochemical staining for MMR proteins. Immunohistochemical staining for MLH1, PMS2, MSH2, and MSH6 was performed on tumor samples of the proband **(a)** and her parents (**b** and **c**). Scale bar is 20 μm.

screening recommendations. Here, we present a case of LS with germline mutations in two different MMR genes inherited from two LS families. Notably, endometrial cancer developed at 37, an unusual age for LS-associated cases.

2. Case presentation

A 45-year-old female, previously in good health with a body mass index of 23.6 and regular menstruation cycles, experienced progressive menorrhagia and dizziness eight years ago. Initial assessment revealed iron deficiency anemia. A transvaginal ultrasound identified a 5.5 × 4.8 × 4.6 cm endocervical mass, leading to a biopsy confirming endometrioid adenocarcinoma. Pelvic magnetic resonance imaging, performed for staging, revealed a 5.5 × 5.0-cm tumor involving the endometrium with invasion more than 50 % of the myometrial thickness and extension to the endocervix (Supplementary Figure S1). Considering the cervical involvement, modified radical hysterectomy was included in the staging surgery, and histological analysis demonstrated grade 3 endometrial endometrioid adenocarcinoma, stage II (pT2N0M0) per International Federation of Gynecology and Obstetrics criteria. After recovery from the surgery, she received adjuvant radiotherapy with a total dose of 4,500 cGy in 25 fractions. Two years after surgery, a recurrent 6.2 × 4.5-cm tumor, located at the left pelvis involving the left ovary and sigmoid colon, was detected by abdominal computed tomography. Complete optimal debulking surgery, including excision of the recurrent tumor and bilateral adnexa with segmental resection of involved sigmoid colon, was performed, followed by six cycles of chemotherapy with paclitaxel, doxorubicin, and cisplatin. However, disease progressed. A peritoneal seeding tumor at the greater omentum and a metastatic tumor at left pelvic wall with several enlarged nodules at mesentery developed soon after completion of the chemotherapy. Considering the young age, good performance status, limited metastases, and potential survival benefit of complete cytoreduction, surgical treatment was offered again. After understanding the potentially increased complexity of surgery by prior radiotherapy, the patient underwent another debulking surgery. The tumors at greater omentum and left pelvic wall were completely resected, but the mesenteric nodules could not be removed due to adhesion to superior mesenteric artery, leading to a suboptimal debulking surgery. After recovery, the patient received six cycles of chemotherapy with ifosfamide and carboplatin. The abdominal computed tomography performed following 6 months of ifosfamide and carboplatin showed progressive disease with a new peritoneal seeding tumor at greater omentum and mildly enlarged mesenteric nodules (Supplementary Figure S2). IHC staining of the most recent resected tumor specimen revealed preserved MSH2 but loss of MLH1, PMS2, and MSH6 (Fig. 1a). Four out of the five microsatellite markers—BAT-25, BAT-26, NR-21, and NR-27—showed instability, indicating MSI-H (Table 1). Subsequently, the patient was referred to a medical oncologist and enrolled in a phase 1 clinical trial investigating the safety and efficacy of a new anti-PD-L1 antibody (LY3300054) for advanced solid tumors. The tumor responded well to the anti-PD-L1 antibody. The seeding tumor at greater omentum could not be identified at 1st response evaluation, which was four cycles (8 weeks) after treatment, and the size of mesenteric nodule decreased from 2.1 cm to 0.7 cm

Table 1
Cancer history and status of DNA mismatch repair.

Family members	Subject number	Sex	Cancer type (age at diagnosis)	Loss of MMR	MSI	Germline mutation
Proband	III-6	F	Endometrial cancer (36)	MLH1 PMS2 MSH6	High	*MLH1* (p. R226X) *MSH6* (p. R911X)
Father	II-7	M	CRC (63)	MLH1 PMS2	High	*MLH1* (p. R226X)
Mother	II-8	F	CRC (40)	MSH6	Stable	*MSH6* (p. R911X)
Grandfather (paternal)	I-1	M	CRC	N/A	N/A	N/A
Aunt (paternal)	II-1	F	Pancreatic cancer	N/A	N/A	N/A
Aunt (paternal)	II-2	F	Pancreatic cancer	N/A	N/A	N/A
Uncle (paternal)	II-3	M	CRC (72)	N/A	N/A	*MLH1* (p. R226X)
Cousin (paternal)	III-3	F	CRC (46)	MLH1 PMS2	High	*MLH1* (p. R226X)

CRC, colorectal cancer; F, female; M, male; MMR, mismatch repair protein; MSI, microsatellite instability; NA, not available.

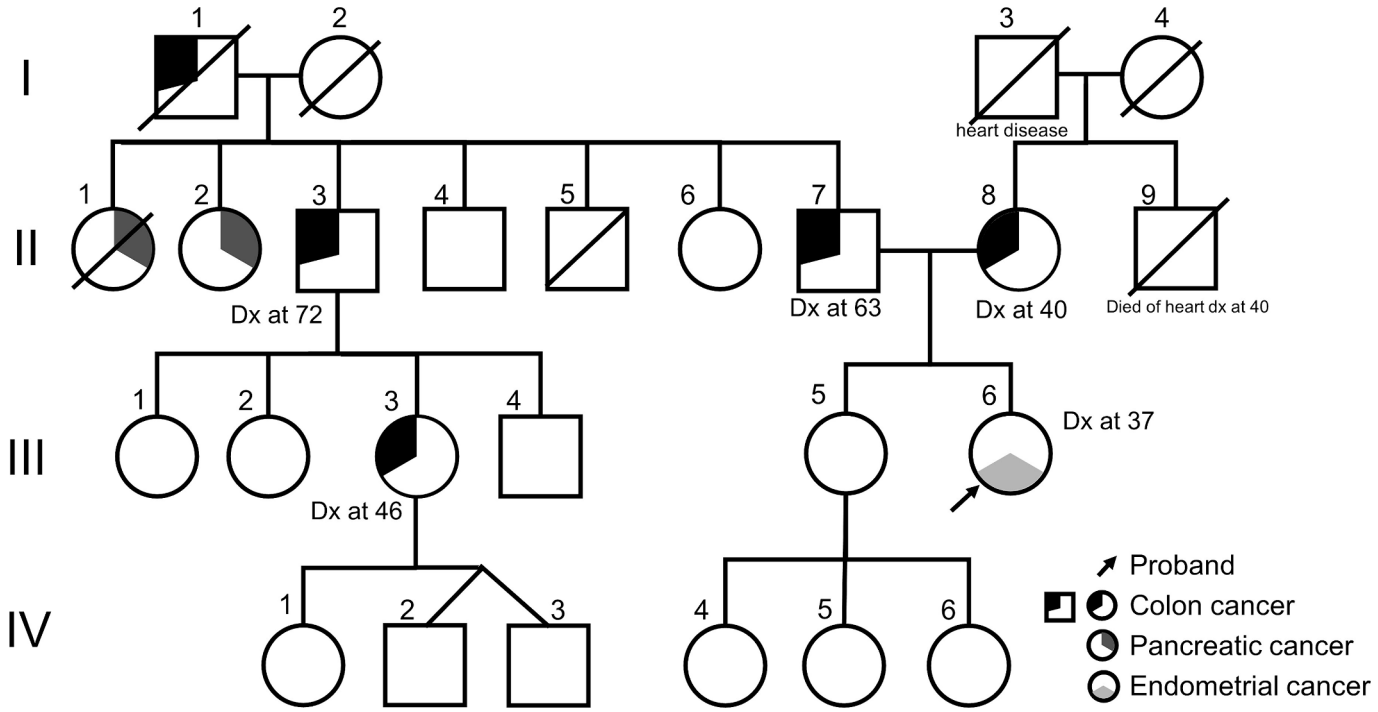

Fig. 2. Pedigree of the family. The proband (III-6) is indicated by an arrow. Individuals I-1, II-3, II7 (proband's father), and II-8 (proband's mother) had colon cancer, and individuals II-1 and II-2 had pancreatic cancer. The diagonal line indicates that the individual is now deceased.

(Supplementary Figure S2). After 46 months, a near-complete response was achieved. At present, the treatment has been discontinued due to the termination of drug development, and the patient is under active surveillance.

After the referral, the detailed family history was obtained (Fig. 2 and Table 1). The patient's father (II-7) and mother (II-8) had ascending colon cancer at the age of 63 and 40, respectively. Her mother reported no family history of cancer, while multiple family members of her father do. Her father's brother had colon cancer at the age of 72 (II-3), and two sisters (II-1 and II-2) had pancreatic cancer. Her grandfather (I-1) and cousin from her father's side (III-3) also had colon cancer. This pattern fulfills the Amsterdam diagnostic criteria for Lynch syndrome. The newer guidelines from the Society of Gynecologic Oncology (SGO) and National Comprehensive Cancer Network (NCCN) also indicate the diagnosis of endometrial cancer at age of 37 meets the criteria for testing of Lynch syndrome. Exome sequencing (ES) was performed on the genomic DNA extracted from the patient's peripheral blood, and the results showed a pathologic variant in *MLH1* (c.676C > T, p.R226X) and *MSH6* (c.2731C > T, p.R911X) (Supplementary Figure S3). Both mutations are reported as pathogenic in the ClinVar database. When the primary tumor sample of her father was analyzed, IHC staining showed loss of MLH1 and PMS2 with preserved MSH2 and MSH6 (Fig. 1b), and only the *MLH1* c.676C > T (p.R226X) was detected by ES without any *MSH6* mutations (Supplement S3). To determine whether the *MSH6* c.2731C > T is a de novo or inherited mutation, the status of MMR and MSI in the tumor sample of her mother was examined. Although none of the five microsatellite markers showed instability (Table 1), loss of MSH6 was found by IHC staining, with preserved MLH1, PMS2, and MSH2 (Fig. 1c). Subsequently, ES on germline DNA revealed a truncating mutation in *MSH6* (Supplementary Figure S3), which is the same *MSH6* mutation detected in the patient. Therefore, this patient was conclusively diagnosed with Lynch syndrome, harboring germline mutations in both *MLH1* and *MSH6*, of paternal and maternal origin respectively.

3. Discussion and conclusions

Deficient MMR tumors typically exhibit loss of expression in one of the MMR heterodimers, either MLH1/PMS2 or MSH2/MSH6, or isolated loss of PMS2 or MSH6. Concurrent loss of expression in proteins from both MMR heterodimers is uncommon (Wang et al., 2018; Moreno et al., 2020; de Freitas et al., 2023). In this case, we present an endometrial cancer patient from a LS family with a *MLH1* c.676C > T mutation. In addition to the expected loss of MLH1/PMS2 expression, IHC staining on the tumor sample incidentally revealed an additional loss of expression in MSH6. ES on genomic DNA from blood identified an *MSH6* c.2731C > T mutation. Subsequent confirmation showed that this mutation was inherited from her mother, who had early-onset ascending colon cancer despite no cancer family history. The unusual pattern of MMR loss underscores the importance of routine assessment of MMR protein expression in patients with endometrial cancer. Moreover, diagnosing LS in a case without a cancer family history, such as the patient's mother, emphasizes the necessity of genomic testing for patients when dMMR is detected, because the negative family history could be caused by the small families, lack of knowledge of the family history, and the death of relatives at a relatively young age from non-cancer causes. Additionally, the diagnosis of endometrial cancer before the age of 40 is uncommon for LS-related endometrial cancer, suggesting that earlier cancer screening may be warranted for patients carrying two MMR mutations.

Cases with concurrent loss of expression in proteins from both MMR heterodimers have been reported in various studies (Wang et al., 2018; Moreno et al., 2020; de Freitas et al., 2023; Stelloo et al., 2017). This uncommon MMR protein expression pattern, observed in less than 5 % of all dMMR tumors, is mostly documented in gastrointestinal tract tumors (Wang et al., 2018; Moreno et al., 2020). A recent study by Freitas et al (Stelloo et al., 2017) reported a relatively high incidence of concurrent loss of MMR heterodimers in endometrial carcinoma. Out of 116 cases, 15 (12.9 %) showed loss of both MLH1/PMS2 and MSH2/MSH6. However, this elevated occurrence of unusual MMR protein expression in endometrial cancer contrasts with findings from prior studies (Wang

et al., 2018; de Freitas et al., 2023). Therefore, further research is necessary to clarify the frequency of concurrent loss of MMR heterodimers in endometrial cancer.

The loss of MMR protein expression can result from either germline mutations in one of the MMR genes or acquired inactivation of MMR protein expression. Previous studies have indicated that most cases with concurrent loss of expression in proteins from both MMR heterodimers were caused by the epigenetic silencing of the *MLH1* gene and somatic mutations in the other MMR gene (Wang et al., 2018). In some instances, germline mutation in one MMR gene was followed by somatic mutations affecting the second MMR gene (Moreno et al., 2020). While Yilmaz et al (Yilmaz et al., 2020) reported the presence of germline mutations in two different MMR genes leading to the concurrent loss of proteins in MMR heterodimers, it remained unclear whether the germline mutation was inherited from the maternal, paternal, or was a de novo mutation. In our case, germline sequencing analysis identified a well-known pathogenic variant in *MLH*1 and *MSH6*, respectively. The patient is a member of LS family with an *MLH1* mutation. Further genomic analysis of blood from her mother, who did not have any family history of cancer, confirmed the diagnosis of LS and the origin of the *MSH6* mutation. To our knowledge, this is the first case demonstrating LS with germline mutations in two different MMR genes simultaneously, inherited from two LS families.

Cancer risks linked to germline mutations in the four MMR genes exhibit notable differences. The cumulative incidence of any cancer by age 70 is higher for *MLH1* (72 %) and *MSH2* (72 %) mutation carriers, while it is lower for those with *PMS2* (18 %) and *MSH6* (54 %) mutations (Møller et al., 2017). Specifically, the cumulative incidence of endometrial cancer by age 70 is 34 %, 24 %, 51 %, and 49 % for carriers of *MLH1*, *PMS2*, *MSH2*, and *MSH6* mutations, respectively (Møller et al., 2017). Although lifetime risks for endometrial cancer associated with MMR gene mutations are high, the risks do not see a significant increase until after the age of 40. Cumulative risks in *MLH1* and *MSH6* mutation carriers at age 40 are relatively low, standing at 3 % and 2 %, respectively (Bonadona et al., 2011; Møller et al., 2017; Dominguez-Valentin et al., 2023). In our case, germline mutations in both *MLH1* and *MSH6* genes were detected, and endometrial cancer manifested at the age of 37, which is younger than the median age at diagnosis for LS-related endometrial cancer. The co-occurrence of *MLH1* and *MSH6* mutations may contribute to this earlier onset of endometrial cancer, suggesting that earlier screening should be considered for patients with concurrent germline mutations in different MMR genes.

CRediT authorship contribution statement

Yi-Ching Huang: Writing – original draft, Visualization, Investigation. **Peng-Chan Lin:** Supervision, Formal analysis, Conceptualization. **Pei-Ying Wu:** Writing – review & editing. **Nai-Syuan Chen:** Writing – review & editing, Resources. **Meng-Ru Shen:** Funding acquisition. **Yu-Min Yeh:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Funding acquisition. **Ya-Min Cheng:** Writing – review & editing, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank the patient and her family for their cooperation for allowing the publication of the manuscript and any accompanying images.

Data Sharing and Data Accessibility.

The data that supports the findings of this study are available in the supplementary material of this article.

Funding information.

This work was funded from the Ministry of Health and Welfare (MOHW112-TDU-B-221-124005) to Meng-Ru Shen, and National Cheng Kung University Hospital (NCKUH-11210014) to Yu-Min Yeh.

Ethics approval.

This study was approved by the Institutional Review Board of National Cheng Kung University Hospital NCKUH (B-ER-110-418, A-EC-112-033). Written informed consent was obtained from the patient.

Patient consent statement.

Written informed consent for publication of their clinical details and clinical images was obtained from the patient. A copy of the consent form is available.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gore.2024.101381.

References

Bonadona, V., Bonaïti, B., Olschwang, S., Grandjouan, S., Huiart, L., Longy, M., et al., 2011. Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. Jama. 305 (22), 2304–2310.

de Freitas, D., Aguiar, F.N., Anton, C., de Almeida, D.C., Bacchi, C.E., Carvalho, J.P., et al., 2023. Clinicopathological characteristics of endometrial carcinomas according to DNA mismatch repair protein status. Heliyon. 9 (6), e17495.

Dominguez-Valentin, M., Haupt, S., Seppälä, T.T., Sampson, J.R., Sunde, L., Bernstein, I., et al., 2023. Mortality by age, gene and gender in carriers of pathogenic mismatch repair gene variants receiving surveillance for early cancer diagnosis and treatment: a report from the prospective Lynch syndrome database. EClinicalMedicine. 58, 101909.

Lynch, H.T., Snyder, C.L., Shaw, T.G., Heinen, C.D., Hitchins, M.P., 2015. Milestones of Lynch syndrome: 1895–2015. Nat Rev Cancer. 15 (3), 181–194.

Møller, P., Seppälä, T., Bernstein, I., Holinski-Feder, E., Sala, P., Evans, D.G., et al., 2017. Cancer incidence and survival in Lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective Lynch syndrome database. Gut. 66 (3), 464–472.

Moreno, E., Rosa-Rosa, J.M., Caniego-Casas, T., Ruz-Caracuel, I., Perna, C., Guillen, C., et al., 2020. Next generation sequencing to decipher concurrent loss of PMS2 and MSH6 in colorectal cancer. Diagn Pathol. 15 (1), 84.

Network NCC. endometrial Cancer (Version 1.2024) 2023 [Available from: https://www.nccn.org/professionals/physician_gls/pdf/uterine.pdf.

Stelloo, E., Jansen, A.M.L., Osse, E.M., Nout, R.A., Creutzberg, C.L., Ruano, D., et al., 2017. Practical guidance for mismatch repair-deficiency testing in endometrial cancer. Ann Oncol. 28 (1), 96–102.

Wang, T., Stadler, Z.K., Zhang, L., Weiser, M.R., Basturk, O., Hechtman, J.F., et al., 2018. Immunohistochemical null-phenotype for mismatch repair proteins in colonic carcinoma associated with concurrent MLH1 hypermethylation and MSH2 somatic mutations. Fam Cancer. 17 (2), 225–228.

Yilmaz, A., Mirili, C., Bilici, M., Tekin, S.B., 2020. Colorectal cancer in Lynch syndrome associated with PMS2 and MSH6 mutations. Int J Colorectal Dis. 35 (2), 351–353.