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Novel germline mutations in MLH1 and PMS2 in familial Lynch syndrome

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To the editor:

Lynch syndrome (LS), an autosomal dominant inherited syndrome causing tumor predisposition, is often associated with colorectal cancer (CRC) and, second most commonly, endometrial cancer (EC).[1–3] It is caused by a heterozygous (one allele affected) inactivating germline mutation in one of the mismatch repair (MMR) genes; germline deletion of the epithelial cell adhesion molecule (EPCAM) gene may also result in LS.[4–6] Here, we report a Chinese family with LS, identifying two novel mutations in MMR (mismatch repair) genes that are related to LS development and may facilitate its diagnosis.

A 49-year-old woman was diagnosed with uterine clear cell carcinoma and received laparoscopic bilateral hystero-salpingo-oophorectomy in the Department of Obstetrics and Gynecology of Peking University Third Hospital in 2017. Immunohistochemistry (IHC) of the patient's tumor tissue was negative for mutL homolog1 (MLH1) and postmeiotic segregation increased 2 (PMS2) protein (Fig. 1), and no MLH1 methylation was detected, indicating defective DNA mismatch repair (dMMR) machinery. The patient met the screening principles of Chinese Society of Clinical Oncology (CSCO) for LS genetic testing (Fig. 2).[7] In addition to the patient (III1), her parents (II2, II3), aunt (II4), aunt's husband (II5) and aunt's daughter (patient's cousin, III5), as well as the patient's uncle (II6), sister (III4), and daughter (IV1), were included in this study. The patient's father, paternal grandmother, paternal aunt, and paternal uncle were diagnosed with colorectal cancer at the ages of 66, 70, 33, and 49 years, respectively. Neoplasm recurrence occurred in the patient's paternal aunt at the age of 66 years. In addition, the patient's female cousin was diagnosed with endometrial cancer at the age of 38 years. All these diagnoses were verified by pathological examination. The patient's family history (Fig. 3) met the Amsterdam II criteria for LS (Table 1).[8]

Peripheral blood samples (10 ml) were collected from the patient and her family members. Blood cells were isolated from the plasma, and DNA was extracted. After DNA isolation, whole-exome sequencing of 549 genes related to genetic predisposition to malignant tumors was performed and analyzed. No mutations in the v-raf murine sarcoma viral oncogene homolog B1 (BRAF) gene or other known mutations in Lynch-relevant genes were detected. However, previously unidentified mutations, including point mutations at exon 7 of epithelial cell adhesion molecule (EPCAM) (c.C790G), exon 7 of postmeiotic segregation increased 2 (PMS2) (c.748T>C), exon 39 of tuberous sclerosis2 (TSC2) (c.A4979G) and exon 39 of TSC2 (c.C5008A) and a frameshift mutation at exon 6 of MLH1 (mutL homolog1) (c.475_476del), were identified in this patient and considered potential disease-causing mutations. Among the patients with endometrial carcinoma, the total percentages of samples with MLH1 and PMS2 mutations were 5.23% and 3.79%, respectively. Consistently, two gene mutations were present in this patient: a point mutation at exon 7 of PMS2 (c.748T>C) and a frameshift mutation in exon 6 of MLH1 (c.475_476del), which were also detected in the patient's father and aunt, respectively. In addition, we found a frameshift mutation at exon 6 of MLH1 (c.475_476del) in the patient's uncle and a point mutation at exon 7 of PMS2 (c.748T>C) in this patient's cousin. We made further efforts to examine the effects of the two novel mutations in PMS2 and MLH1 on protein structure by analysis in Pfam, a database of protein families, and SWISS-MODEL. The results showed that the point mutation at exon 7 of PMS2 (c.748T>C) is in the DNA mismatch repair domain (PMS2:p.S250P) (Fig. 4A) and leads to a slight change in the amino acid side chain, with serine replaced by proline (Fig. 4B and C). This change might not interfere with the DNA mismatch repair function of PMS2. However, if an important site is affected, this small change might have a large impact. The other mutation, a frameshift at exon 6 of MLH1 (c.475_476del/MLH1:p. I159fs*12), resulted in a truncated protein that had no DNA mismatch repair or MLH1_C domains (Fig. 5A) and thus fundamentally disrupted the MLH1 protein structure (Fig. 5B and C).

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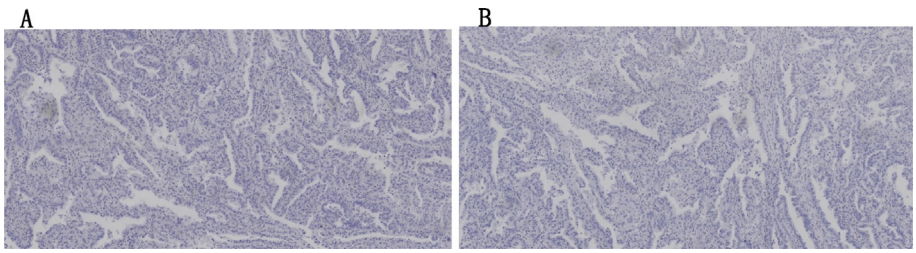

Fig. 1. Immunohistochemical staining in the patient's tumor tissue. A.MLH1 expression is missed in the tumor tissue. B.PMS2 expression is missed in the tumor tissue.

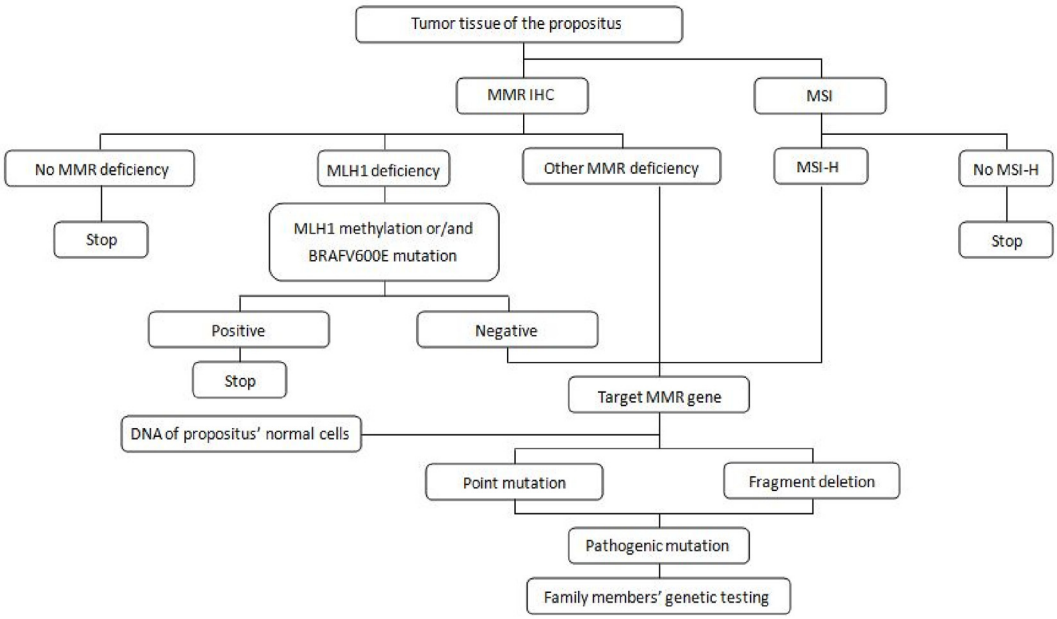

Fig. 2. Screening principles of the Chinese Society of Clinical Oncology (CSCO) for LS genetic testing.

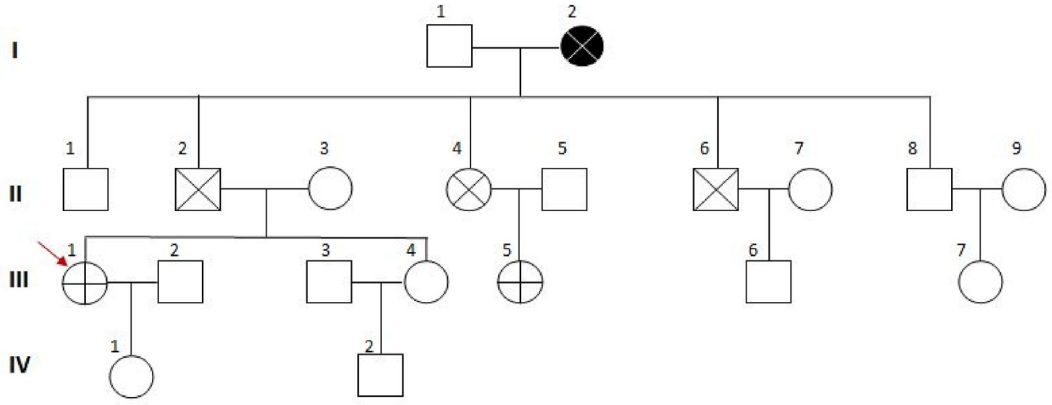

Fig. 3. Pedigree of the patient's family. Squares represent males, and circles represent females. The arrow denotes the patient, crosses denote individuals diagnosed with cancer, and the black color denotes deceased patients. × , colorectal carcinoma; ┼, endometrial carcinoma.

Table 1
Amsterdam II criteria.

There should be at least three relatives with Lynch/HNPCC-associated cancer (cancer of the colorectum, endometrium, small bowel, ureter or renal pelvis)
One should be a first-degree relative of the other two
At least two successive generations should be affected
At least one should be diagnosed before age 50
Familial adenomatous polyposis should be excluded
Tumors should be verified by pathological examination

It is highly possible that the MLH1 mutation (c.475_476del/MLH1:p.I159fs*12) is the driver mutation in LS disease development. Therefore, it was reasonable to speculate that there is a causative relationship between these two mutations and Lynch syndrome (Table 2).

Notably, among the family members without tumors, only a frameshift mutation at exon 6 of MLH1 (c.475_476del) was detected in the patient's daughter. No disease-causing gene mutations were found in the patient's mother, sister, or husband. According to these results, it is highly possible that both genetic mutations were derived from the

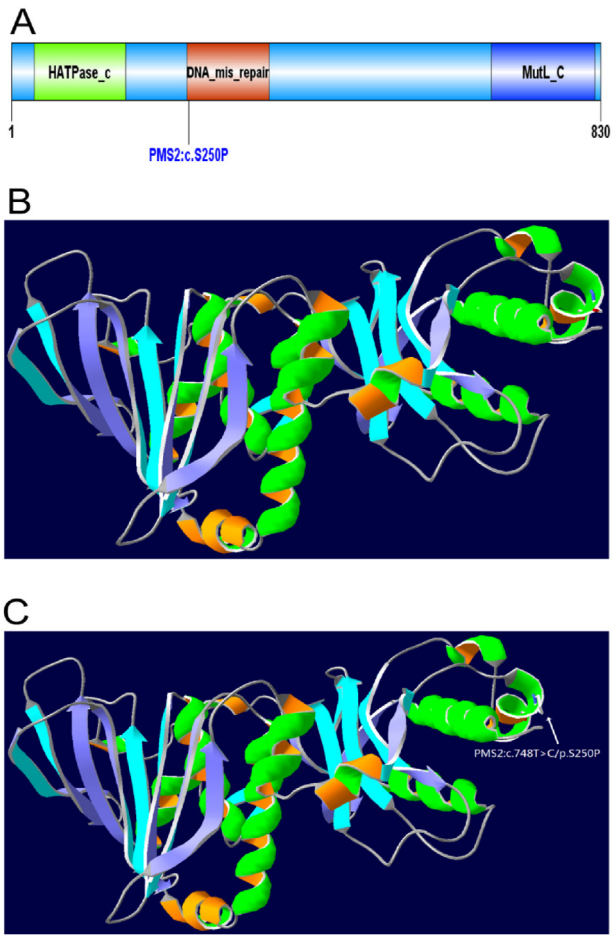

Fig. 4. Protein domains map and 3D structure of PMS2. A.PMS2 protein domains map. B.3D structure map of PMS2 wild type. C.3D structure map of PMS2 with mutant T748C. Arrow represents p.s250p mutation.

paternal line.

In the present study, two novel mutations in the MLH1 and PMS2 genes were identified in a Chinese family with LS. We consider that the two new MMR mutations contribute significantly to LS occurrence based on the evidence listed below. First, according to clinical signs and symptoms, the patient and her family met the screening principles of CSCO for LS genetic testing and Amsterdam criteria II, respectively. Second, various mutations at PMS2 and/or MLH1 have been identified and have critical functions in the development of EC or CRC. Third, MutL alpha, the key component of the postreplicative DNA mismatch repair system, consists of PMS2 and MLH1. Bioinformatics analyses of the protein domain and structure indicate that the two mutations cause remarkable changes in protein structure, either protein folding alterations or protein truncation. In particular, the frameshift mutation at exon 6 of MLH1 (c.475_476del) is predicted to be the driver mutation predisposing the patient to LS disease. Nevertheless, further investigation of the effect of the two new mutations on PMS2 or MLH1 protein levels and their function, particularly in tumorigenesis, is required to support our speculation that they are causative mutations.

Among members of the patient's family, an uncle with the MLH1 mutation had CRC (colorectal cancer), and a cousin with the PMS2 mutation had EC. Other family members harboring both mutations developed only one kind of cancer as well. Thus, long-term observation and follow-up are necessary to monitor the development of existing and/or new cancer in all family members. Notably, the patient's daughter has the MLH1 mutation but has not developed any type of cancer thus far; however, the inherited genetic abnormality and family history of LS strongly increase her susceptibility to CRC and/or EC, especially EC.

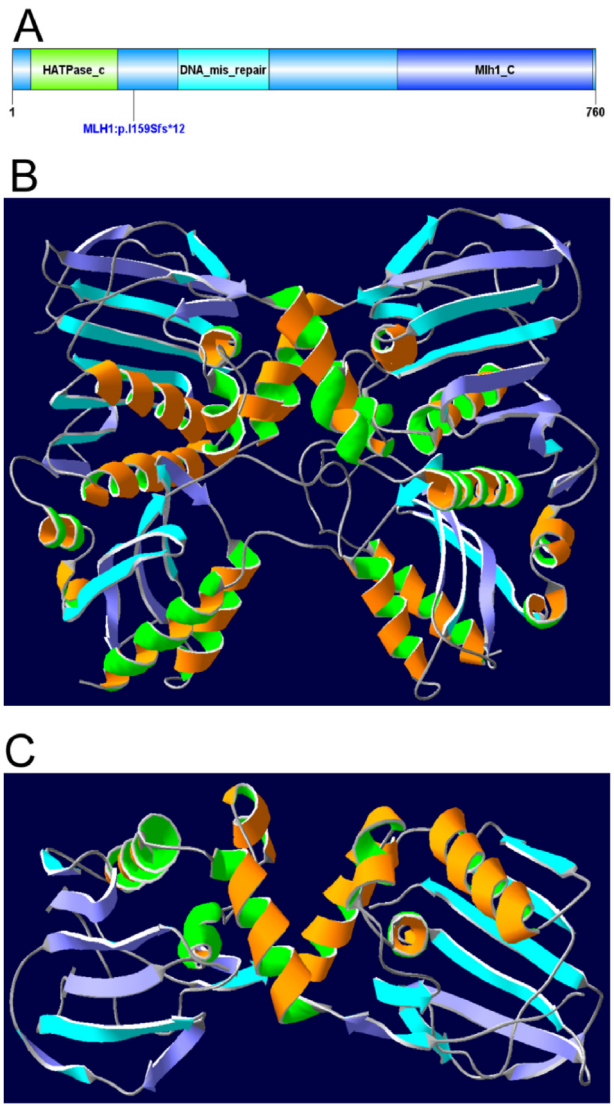

Fig. 5. Protein domains map and 3D structure of MLH1. A.MLH1 protein domains map. B.3D structure map of MLH1 wild type. C.3D structure map of MLH1 with mutant 475_476del.

Thus, in the future, special attention should be given to her health, especially any issues arising from a potential malignancy. The purpose of performing genetic testing in an LS family is to evaluate the risk of tumorigenesis, provide health education, and detect cancer at a very early stage. Accumulating evidence indicates that all members of families with LS who are 20–25 years old or older require proper medical examination for CRC and EC and other extracolonic cancers. Colonoscopy screening should be started at the age of 20–25 years and performed at least every 3 years.[9] After 35 years of age, an annual colonoscopy screen is necessary. In addition, for 30- to 35-year-old females in families with LS, annual gynecologic surveillance, including transvaginal ultrasonography, CA125 detection, and Pap smear, is recommended.[10,11] MMR deficiencies were detected in 34.9% of endometrial cancer patients; as a result, Lynch syndrome screening with MMR IHC should be considered in all patients regardless of personal or family history of Lynch syndrome-related cancer.[12]

In summary, we identified novel mutations in the PMS2 and MLH1 genes that might be pathogenic in a Chinese family with LS. The point mutation at exon 7 of PMS2 and the frameshift mutation at exon 6 of MLH1 were considered to play an important role in LS development. In the future, our findings may help with the early diagnosis and routine management of persons in families with lynch syndrome.

Table 2
Gene mutations found in different family members in this pedigree.

	Type of cancer	Gene	Mutation type	Transcript	Exon	DNA base change	Amino acid change	Polyphen2_HDIV	SIFT	Polyphen2_HIVAR
III1	Endometrial	EPCAM	point mutation	NM_002354	exon7	c.C790G	p.L264V	D	D	D
		MLH1	frameshift mutation	NM_000249	exon6	c.475_476del	p.I159fs	–	–	–
		PMS2	point mutation	NM_000535	exon7	c.T748C	p.S250P	D	T	P
		TSC2	point mutation	NM_001077183	exon39	c.A4979G	p.H1660R	D	T	D
		TSC2	point mutation	NM_001077183	exon39	c.C5008A	p.P1670T	D	T	D
II2	Colorectal	MLH1	frameshift mutation	NM_000249	exon6	c.475_476del	p.I159fs	–	–	–
		PMS2	point mutation	NM_000535	exon7	c.T748C	p.S250P	D	T	P
II4	Colorectal	PMS2	point mutation	NM_000535	exon7	c.T748C	p.S250P	D	T	P
		MLH1	frameshift mutation	NM_000249	exon6	c.475_476del	p.I159fs	–	–	–
		MITF	point mutation	NM_000248	exon3	c.A302G	p.E101G	D	T	D
		SDHA	point mutation	NM_001294332	exon13	c.A1742T	p.Y581F	B	T	B
		MET	point mutation	NM_000245	exon2	c.T1027C	p.F343L	P	D	P
II6	Colorectal	MLH1	frameshift mutation	NM_000249	exon6	c.475_476del	p.I159fs	–	–	–
III5	Endometrial	MITF	point mutation	NM_000248	exon3	c.A302G	p.E101G	D	T	D
		PMS2	point mutation	NM_000535	exon7	c.T748C	p.S250P	D	T	P

SIFT and Polyphen2 are gene function prediction programs. In SIFT, T means tolerated, D means deleterious. B means benign, P means possibly damaging.
Note: EPCAM: epithelial cell adhesion molecule; MLH1: mutL homolog1; PMS2: postmeiotic segregation increased 2; TSC2: tuberous sclerosis2; MITF:microphthalmia-associated transcription factor; SDHA:recombinant succinate dehydrogenase complex subunit A MET:cellular-mesenchymal to epithelial transition factor.

Author contributions

CY: Wrote the manuscript and prepared the figures. CX and LQ: Prepared the figures and ethic. LC: Provided HE staining for tumor tissue, LH: Designed, analyzed and revised the manuscript. All authors approved the final version of the manuscript as current form.

Ethics approval and consent

This research was approved by the Peking University Third Hospital ethics committee (S2019374). Written informed consent was obtained from individual or guardian participants.

Consent for publication

All authors approved the final version of the manuscript as current form.

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Declaration of competing interest

The authors declare there is no conflicts of interest regarding the publication of this paper.

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