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MutL homolog 1 germline mutation c.(453+1_454-1)_(545+1_546-1)del identified in lynch syndrome: A case report and review of literature

Zhang XW *et al*. *MLH1* germline mutation identified in LS

Abstract

BACKGROUND

Lynch syndrome (LS) is an autosomal dominant hereditary disorder because of germline mutations in DNA mismatch repair genes, such as MutL homolog 1 (MLH1), PMS1 homolog 2, MutS homolog 2, and MutS homolog 6. Gene mutations could make individuals and their families more susceptible to experiencing various malignant tumors. In Chinese, *MLH1* germline mutation c.(453+1_454-1)_(545+1_546-1)del-related LS has been infrequently reported. Therefore, we reported a rare LS patient with colorectal and endometrioid adenocarcinoma and described her pedigree characteristics.

CASE SUMMARY

A 57-year-old female patient complained of irregular postmenopausal vaginal bleeding for 6 mo. She was diagnosed with LS, colonic malignancy, endometrioid adenocarcinoma, secondary fallopian tube malignancy, and intermyometrial leiomyomas. Then, she was treated with abdominal hysterectomy, bilateral oviduct oophorectomy, and sentinel lymph node resection. Genetic testing was performed using next-generation sequencing technology to detect the causative genetic mutations. Moreover, all her family members offered a free genetic test, but no one accepted it.

CONCLUSION

No tumor relapse or metastasis was found in the patient during 30 mo follow-up period. The genetic panel sequence showed a novel pathogenic germline mutation in *MLH1* c.(453+1_454-1)_(545+1_546-1)del for LS. Moreover, cancer genetic counseling and testing are still in the initial development state in China, and maybe face numerous challenges in the further.

Key Words: Lynch syndrome; Colorectal cancer; Endometrial cancer; *MLH1* gene; Gene testing; Case report

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Core Tip: Lynch syndrome (LS) is an autosomal dominant hereditary disorder because of germline mutations in DNA mismatch repair genes, such as MutL homolog 1 (MLH1) gene, PMS1 homolog 2 gene, MutS homolog 2 gene, and MutS homolog 6 gene, which makes the patient more susceptible to other malignancies. In Chinese, *MLH1* germline mutation c.(453+1_454-1)_(545+1_546-1)del-induced LS has been infrequently reported. In the present study, we found a rare LS patient with colorectal and endometrioid adenocarcinoma. Moreover, the genetic panel sequence showed a novel pathogenic germline mutation in *MLH1* c.(453+1_454-1)_(545+1_546-1)del for LS.

INTRODUCTION

Lynch syndrome (LS) is an autosomal dominant inherited disorder because of germline mutations in DNA mismatch repair (MMR) genes, such as MutL homolog 1 (MLH1), PMS1 homolog 2 (PMS2), MutS homolog 2 (MSH2), and MutS homolog 6 (MSH6), which makes the patient more susceptible to other malignancies[1,2]. *MLH1*, *MSH2*,

MSH6, and *PMS2* in LS account for approximately 50%[3-5], 40%[5], 7%-20%[3-8], and < 6%[3,4,9], respectively. Additionally, specific MMR gene deficiencies might result in a different age of onset, types of malignancy, and clinical signs[10]. The *MLH1* gene defects could decrease *MLH1* protein, affecting the MMR function of DNA, leading to errors in DNA replication and ultimately inducing neoplams[11,12].

LS can be classified as type I and II according to the location of tumors[3,13,14]. Type I is an intestinal neoplasm, such as colorectal cancer[14]. Besides, type II is defined as colorectal malignancy complicated with parenteral cancers, including gastric cancer[15], renal cell cancer[16], epithelial ovarian cancer[17], endometrial cancer[2], bladder cancer[18,19], breast cancer[20] and even repeated stroke[10]. Endometrial cancer is the most frequent parenteral tumor among LS patients[21,22], which ranks 3th in the mortality of all gynecological cancers[23]. In recent years, Lynch syndrome-associated endometrial cancer (LSAEC) has received increasing attention in the medical field[24]. Furthermore, the offspring of LS patients will have a 50% incidence of inheritance[24]. More than 2600 mutations have been reported worldwide[10,24,25], but *MLH1* exon 6 (c.(453+1_454-1)_(545+1_546-1)del-induced LS has been rarely described in Chinese. Therefore, we presented a rare case with an *MLH1* germline mutation, analyzed her pedigree characteristics, and reviewed the *MLH1* gene mutation loci.

CASE PRESENTATION

Chief complaints

A 57-year-old Chinese female patient complained of irregular postmenopausal vaginal bleeding. The demographic characteristics of the patient are listed in Table 1.

History of present illness

She had the clinical symptoms of irregular vaginal bleeding for 6 mo.

History of past illness

She had a medical history of colon cancer and received a radical colon cancer operation twenty years ago.

Personal and family history

The patient and many of her family members had a cancer history.

Physical examination

A small amount of white and no odor secretions were found in the vagina. A smooth cervical surface was detected. The vulva was atrophy, vaginal was patency, and mucosal fold atrophy was palpated. Moreover, the uterine was in an anterior position, with a smooth surface and a good range of motion. No obvious abnormality was found in the bilateral adnexal area.

Laboratory examinations

No abnormality was found in the blood routine examination.

Imaging examinations

Ultrasound

Preoperative abdominal Doppler ultrasound showed that the uterus with the size of 3.8 cm × 3.5 cm × 3.1 cm was located in the ante-position, the uterine cavity line was clear, the endometrial thickness was 1.1 cm (significantly greater than the normal value of endometrial thickness in postmenopausal women), and the ultrasonic echo of the endometrium was uneven. In addition, bilateral ovaries and adnexa presented no abnormality. Color Doppler flow imaging showed no abnormal blood flow signal.

Magnetic resonance imaging

Abdominal Magnetic resonance imaging showed that the uterus was in an anteversion and flexion position. A mass of equal T1 and slightly long T2 signal shadow was found in the uterine cavity, with unclear boundary (Figure 1A). The tumor size was about 31

mm × 23 mm, and the display of the uterine junction was not clear (Figure 1B and C). The enhanced images showed that the lesions were inhomogeneous enhancement. Diffusion-weighted imaging showed that the lesions were a high signal. The shape and signal of bilateral adnexa were normal (Figure 1D). There was no obvious abnormal signal in the bladder and rectum. No abnormality was found in bilateral iliac vessels and inguinal lymph nodes. No effusion was found in the pelvic cavity, and no obvious abnormal signal was found in pelvic wall soft tissue.

FINAL DIAGNOSIS

The clinical diagnosis was endometrioid adenocarcinoma (IIIA1) and LS.

TREATMENT

After general anesthesia, abdominal hysterectomy and bilateral oviduct oophorectomy were performed. The whole uterus and bilateral appendages were examined during the operation by fast-frozen histopathology. It revealed a poorly differentiated adenocarcinoma of the uterus, which infiltrated the superficial muscularis. Subsequently, sentinel lymph node resection was also performed in this patient. After surgery, the patient was treated with regular chemotherapy for six courses, including paclitaxel (Nanjing Green Leaf Pharmaceutical Co., Ltd., Nanjing, China) and carboplatin injection (Qilu Pharmaceutical Co., Ltd., Jinan, China).

OUTCOME AND FOLLOW-UP

The biopsy histochemical (hematoxylin-eosin) staining showed that endometrial cancer was moderate to poorly differentiated. A few of its lesions were accompanied by squamous differentiation. The tumor infiltrated into the superficial muscle wall. Noticeably, one side of the fallopian tube showed cancerous lesions, while the other side of the fallopian tube and bilateral ovaries showed no cancerous lesions. However, cervical vessels, blood vessels, lymphatic vessels, and nerves were not invaded. Bilateral pelvic lymph nodes were normal.

Immunohistochemical (IHC) staining results of pathological showed MLH1 (-), PMS2 (-), MSH6 (+-), MSH2 (+-), BRAF V600E mutation-specific antibody (VE1) (Ventana IHC enhanced amplification kit) (-), CD31 (-), D2-40 (-), CK5/6 (partial lesions +-), p63 (+-), CDX2 (-) (Figure 2). Besides, the positive rate of PR was 90+ACU- (+-). The positive rate of ER was 90+ACU- (+-). The positive rate of Ki67 was 60+ACU-. P53 was scattered weak positive, and P16 showed partial positive. She was diagnosed with endometrioid adenocarcinoma (IIIA1).

No tumor recurrence or metastasis was found during 2.5 years follow-up visit. Computed tomography scan was performed after six chemotherapy courses and showed no abnormality in the head, liver, gallbladder, spleen, pancreas, bilateral kidney, bilateral ureter, rectum, and lung.

The results of gene sequencing are as follows (Table 3). The deletion mutation of exon 6 del heterozygous was detected in the *MLH1* gene, which was named c.(453+-1+AF8-454-1)+AF8-(545+-1+AF8-546-1)del according to the Human Genome Variation Society (Figure 3). Postoperatively, she was diagnosed with LS, endometrioid adenocarcinoma (IIIA1), colonic malignancy, secondary fallopian tube malignancy, and intermyometrial leiomyomas.

The eldest sister was diagnosed with colon cancer at age 60, the second sister with endometrial cancer at age 60, the third sister with colon cancer at age 40, the older brother with colon polyps three times between the ages of 40 and 50, the mother with endometrial cancer at age 48, and the mother with colon cancer at age 50. The prevalence spectrum of the four generations of patients is shown in Figure 4.

Genetic counseling was conducted among the family relatives. Moreover, we provide free Sanger mutation site verification tests for family members of patients. However, all relatives refused to be tested.

DISCUSSION

Colorectal cancer is the 5th diagnosed cancer in China[23,26]. In 2015, the number of colorectal cancer-related deaths and new cases in China was approximately 191000 and

376300, respectively. Moreover, hereditary colorectal malignancy accounts for 5%-10% of colorectal malignancies, including LS, Li-Fraumeni syndrome, MUTYH-associated polyposis, Juvenile polyposis syndrome, familial adenomatous polyposis, and Peutz-Jeghers syndrome[27]. In this study, the patient had a medical history of colon cancer 20 years ago and has experienced endometrial adenocarcinoma. We found the LS patient carrying a novel pathogenic genetic mutation in *MLH1* deletion. Many researchers have reported diseases caused by *MLH1* gene mutations[28-31]. Hong *et al*[31] detected that the deletion of exon 7 to exon 19 of the *MLH1* gene was a pathogenic mutation causing colorectal cancer. Jia *et al*[28] reported that the p.K618del variant in *MLH1* was the causative pathogenic genetic variant for LS. Solassol *et al*[29] found that an *AluY5a* insertion in *MLH1* exon 6 Led to exon skipping, which induced a pathogenic frameshift in patients who developed colorectal adenocarcinomas. Li *et al*[30] reported that the insertion of a truncated *AluSx* like element into *MLH1* intron 7 results in aberrant splicing and transcription, thus inducing LS. Lagerstedt-Robinson *et al*[32] reported an LS patient caused by the deletion of *MLH1* c.(453+1_454-1)_(545+1_546-1) in Switzerland. However, in China, genetic mutation in *MLH1* c.(453+1_454-1)_(545+1_546-1) del has not been reported. Consequently, we presented a relatively rare LS patient induced by *MLH1* c.(453+1_454-1)_(545+1_546-1)del and described the clinical features, pathological features, and familial morbidity of the proband.

The demographic characteristics of LSAEC are as follows: first, the pathological types are diverse and poorly differentiated; second, the onset age is between 46 and 54 years old; third, the majority of the pathological changes are situated in the lower segment of the corpus uteri[33]. The potential risk of LS patients experiencing another cancer at 10 and 15 years was 25% and 50%, respectively[34]. The present case has colon cancer at age 37. Twenty years later, she was diagnosed with endometrioid adenocarcinoma. The demographic characteristics, in the present patient, were similar to the previous studies[21,34].

Concerning the diagnosis of LS, Amsterdam II[35] and Bethesda[36] criteria have been widely used to screen for LS. In the present study, the patient meets the criteria of the

Amsterdam standard Ⅱ and the revision of the Bethesda guidelines. Nonetheless, the two standards' sensitivity is low because they are based on clinical background and family history[37-39]. Thus, Amsterdam II and Bethesda criteria were inadequate as independent screening tools.

IHC was a useful method for LS screening[37,40,41], particularly in colorectal malignancy patients. The sensitivity and specificity of IHC in patients with MMR mutations are 83% and 89%[42]. When IHC results suggest deleting *MLH1* and *PMS2* proteins, universal screening including *BRAF* testing and *MLH1* promoter methylation analysis is required[10,22,24,25,28,38-40,43,44]. In the present study, the IHC result showed the losses of *MLH1* and *PMS2* proteins, but expressions of *MSH2* and *MSH6* proteins in the tumor cells. Subsequently, *MLH1* mutation was considered. The patient had a medical history of colon cancer and a family history of LS-related cancers. Then, she was diagnosed with LS. Also, we advised the patient and her family members to receive genetic counseling.

Before genetic testing, we provided genetic counseling for the patient and achieved a clear LS family history. We found that the proband's mother (I-1) suffered from primary colon cancer at 55 years and died at 70 years, her sisters (II-1) and (II-3) were affected by colon cancer at 60 years and endometrial cancer at 60 years, respectively, her sister (II-5) experienced colon cancer in 40 years and endometrial cancer in 48 years, her brother (II-7) encountered polyp of colon, and her nephew developed colon cancer at 25 years and died at 27 years. Besides, standard processes of cancer-related genetic counseling should include pre-test counseling, results analysis, and follow-up[28]. In our study, the family history suggested the clinical diagnosis of LS. Then, the patient and family members were given detailed pre-test counseling. However, we cannot make a definitive diagnosis of LS without genetic testing[28]. Consequently, genetic testing was recommended for the proband and her relatives.

Furthermore, our professor used to be willing to pay for all genetic tests for all her family members. Moreover, our corresponding author believes that genetic testing can help at-risk offspring know their risk of developing cancers, thus enabling them to

access personalized precision medicine. Unexpectedly, only the proband received the genetic test, but her family members refused. The reasons for the relatives of the proband to refuse genetic testing are as follows: firstly, they are worried that their genetic problems may cause difficulties in mate selection or affect the stability of marriage; secondly, they are unable to purchase life insurance if they have a genetic defect; thirdly, they are worried about personal privacy exposure. Wang *et al*[45] investigated the willingness and awareness of genetic screening for patients undergoing colon cancer surgery in Peking Union Medical College Hospital who had any protein (*MLH1/MLH2/MLH6/PMS2*) expression deletion suggested by IHC, and the result indicated that 27.4% (61/219) of the patients explicitly refused to undergo genetic screening. The findings of our study and Wang *et al*[45] indicate that gynecologists should strengthen health education. Therefore, cancer genetic counseling and testing are still in the initial development state in China, and maybe face numerous challenges in the further[28]. This dilemma is expected to be improved with better preventative education to the general population and a better understanding of cancer genetics in cancer patients and medical practitioners[28].

The patient achieved positive clinical outcomes during the 30 mo follow-up visit period. However, several limitations exist in this study. Firstly, 6 mo after discharge, the proband's 25-year-old offspring (III-4) was diagnosed with colon cancer and died at age 27. We believe that this unfortunate outcome could have been prevented if her family members had taken genetic testing and then received individualized preventive treatment before the malignant tumor onset. Thus, it is essential to enhance genetic testing awareness among the Chinese population, especially in rural areas.

CONCLUSION

Genetic mutation in *MLH1* exons 6 c.(453+1_454-1)_(545+1_546-1)del is a novel pathogenic mutation of LS in Chinese. This case report emphasizes the value of diagnosis and treatment in patients with inherited malignancy syndromes. To date,

cancer genetic counseling and testing are still in the initial development state in China, and maybe face numerous challenges in the further.

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