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CLINICAL REPORT

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A new mutL homolog 1 c.1896+5G>A germline mutation detected in a Lynch syndrome-associated lung and gastric double primary cancer patient

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Abstract

Background: Mismatch-repair genes (MMRs) ensure high fidelity in genome editing. Loss of function mutation of MMRs will lead to instability of the genome and increase the incidence of cancers. Human mutL homolog 1 (MLH1) is a member of the MMRs, and its mutation is found in Lynch syndrome (LS). In addition to the high incidence of colorectal cancer, LS patients often have other primary cancers. Here, a case of LS-associated lung and gastric double primary cancer was reported.

Methods: Diagnosis of lung and gastric double primary cancer was mainly based on clinical findings, imaging examination, and histopathological data. The tumor tissues and blood samples were collected, and then genomic DNA was extracted and 450 cancer-related gene alteration screening was conducted by next-generation sequencing and the functional verification of a mutant gene was carried out using the PCR method.

Results: We detected the epidermal growth factor receptor L858R, MSH2 R929* and telomerase reverse transcriptase amplification in the lung cancer specimen; CDH1 c.1320+1G>T mutation in the gastric cancer (GC) specimen; and MLH1 c.1896+5G>A germline mutation in the lung and GC specimens by 450 cancer-related gene mutations detection using next-generation sequencing technology. MLH1c.1896+5G>A is a novel germline mutation, and it was verified by the PCR subsequently. It was found that it could affect the splicing of intron 16. Nine relatives of three-generation of the patient were examined and the MLH1 c.1896+5G>A mutation and pedigree analysis were performed. The father's sister without cancer also carries this mutation.

Conclusion: Diagnosis of LS was mainly depended on the following: the cancer histories of his relatives, multi-primary cancers of lung and stomach in his own body, MLH1 and MSH2 gene mutations detected in the cancer tissues. The clinical significance of this new MLH1 c.1896+5G>A germline mutation detected in the LS-associated double primary cancer patient needed further study.

KEYWORDS

germline mutation, Lynch syndrome, mismatch-repair genes, MLH1 c.1896+5G>A

1 | INTRODUCTION

The mutation of mismatch-repair genes (MMRs), as an early molecular event, is involved in the occurrence and development of cancers (Hsieh & Yamane, 2008). MMRs are a group of highly conserved housekeeping genes whose encoded proteins can recognize the mismatched bases and initiate mismatch repair procedures. The decrease in base mismatch repair function caused by the MMRs mutation will increase the instability of the whole genome and lead to a variety of cancer occurrences, such as the stomach, endometrial, ovarian, etc (Hsieh & Yamane, 2008). So far, seven MMR members have been identified in human, including MutL homolog 1 (MLH1; OMIM*120,436), MLH3 (OMIM*604,395), MutS protein homolog 2 (MSH2, OMIM*609,309), MSH3 (OMIM*600,887), MSH6 (OMIM*600,678), PMS1 (OMIM*600,258), and PMS2 (OMIM*600,259) (Larrea, Lujan, & Kunkel, 2010). MMRs mutation is mainly associated with Lynch syndrome (LS), which is a hereditary colorectal cancer (CRC) syndrome and the patients are susceptible to CRC and other malignant tumors (Larrea et al., 2010). LS has been named as hereditary nonpolyposis colorectal cancer (HNPCC) (Lynch et al., 1985), which is to emphasize its heredity and difference from familial adenomatous disease. The clinical features of LS include autosomal dominant inheritance; the average age of cancer onset is young (40–50 year old); multiple primary tumors occur easily (second primary tumors other than CRC occur in 54%–61% patients, and three or more in 15%–23% patients (Larrea et al., 2010). Here, we reported a case of a patient with double primary lesion of lung and gastric cancer (GC), no CRC found. The patient had an obvious family history of cancer. We detected 450 cancer-related gene mutations of the two lesions and blood from the patients using next-generation sequencing (NGS) technique. MLH1 and MSH2 mutation, the most common MMRs mutation in LS, were detected. MLH1 c.1896+5G>A germline mutation has not been reported yet. We concluded that this was a case of LS-associated multiple primary cancers.

2 | CASE PRESENTATION

The patient was a 50-year-old male. In November 2016, he came to the Department of gastroenterology of Nanfang Hospital because of abdominal discomfort and acid regurgitation for 1 year. His father died of lung cancer, and sister had breast cancer and was alive. The gastroscope and pathological examination showed GC possibility (Figure 1, left, a), and the PET-CT images also suggested that there was a large possibility of GC (Figure 1, left, b). In December, "distal gastrectomy" was performed and the postoperation pathological test was GC, ring cell carcinoma (Borrmann III) (Figure 1, left, c). When the patient was admitted to the hospital, the chest positron emission tomography/computed tomography (PET-CT) also reported left pulmonary apex nodule (1.7 × 1.1 cm, SUV max 1.7), considering lung cancer (Figure 1, right, a). After 11 days of stomach surgery, horacoscope-assisted left lobectomy was performed and the postoperation pathological test: the left upper lung infiltrating adenocarcinoma, adherent type mainly (Figure 1, right, b). The patient recovered smoothly and discharged 10 days after the surgeries. Discharge diagnosis: gastric and lung cancer double primary; left lung infiltrating adenocarcinoma (pT1aN0M0, Ia); gastric signet ring cell carcinoma.

Subsequently, formalin-fixed, paraffin-embedded tissue sections of both lung and stomach lesions and matched blood sample as control were sent to Origimed for 450 cancer-related gene mutation detection using NGS method. All the coding exons of the 450 genes and selected introns of 39 genes were captured and sequenced with a mean coverage of 1,000×. Whole exome sequencing with a mean coverage of 200× was performed separately as well. All classes of genomic alterations, including base substitutions, small insertions and deletions (indels), gene amplifications and deletions, gene fusions, and rearrangements, were subsequently analyzed. The results showed that epidermal growth factor receptor (OMIM*131,550) L858R, MSH2 R929* and telomerase reverse transcriptase (TERT, OMIM*187,270) amplification were detected in lung tumor specimens, CDH1 (E-cadherin, OMIM*192,090) c.1320+1G>T was detected in gastric tumor specimens, and MLH1 (GenBank version: NG_007109.2) c.1896+5G>A germline mutation was detected in lung and gastric tumor specimens. The MLH1 c.1896+5G>A has not yet been reported. Then, the PCR of patient's peripheral blood was preformed to verify the MLH1 c.1896+5G>A mutation and affection of it on the clipping RNA. DNA extracted from peripheral blood was analyzed by first generation sequencing and the result was consistent with NGS test that the patient carried MLH1 c.1896+5G>A mutation (Figure 2a). There were two forms observed in the DNA agarose gel electrophoresis: one is intron 16 normal splicing (the band was about 200 bp), and the other band was about 1 KB, because MLH1 c.1896+5G>A made the intron cannot be removed (Figure 2b). This result revealed that MLH1 c.1896+5G>A mutation really interferes with intron 16 splicing. At the same time, the peripheral blood DNA of nine relatives of the patient was tested. Only the relatives of the previous generation (father's elder sister) had MLH1 c.1896+5G>A. The pedigree analysis was shown in Figure 3. This study was approved by the medical ethics committee of Southern Medical University of Nanfang Hospital.

3 | DISCUSSION

With the accumulation of clinical data, it is found that some cancers have family genetic tendencies, that is, the incidence

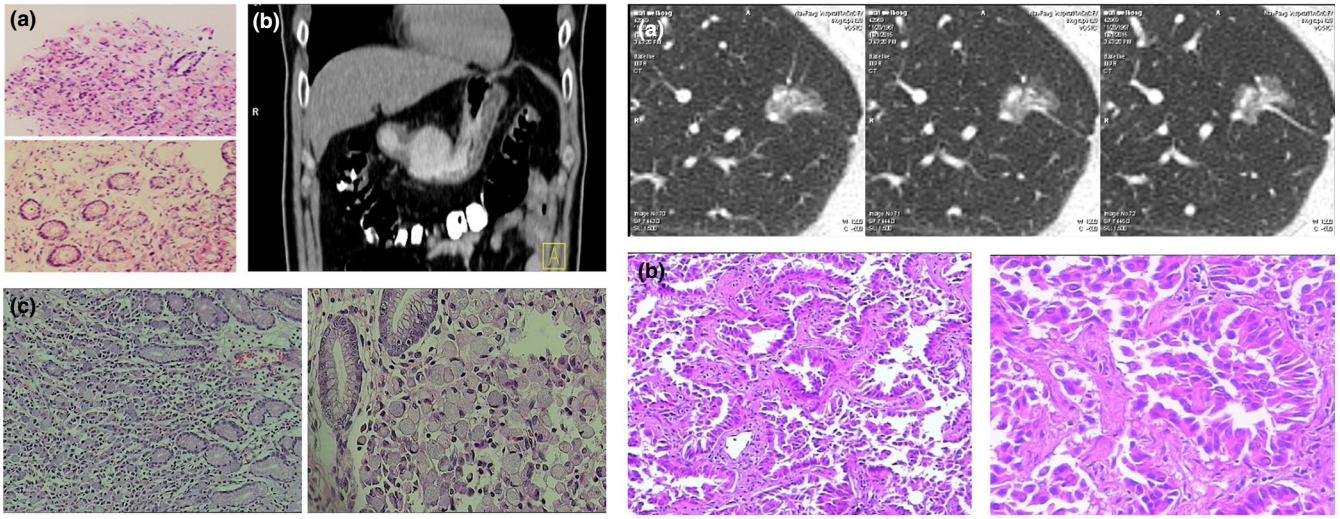

FIGURE 1 The clinical diagnosis of the patient. Left: the gastroscope biopsy pathologic, PET-CT examinations and postoperation pathological test showed the gastric cancer (GC). (a) Gastroscope biopsy pathology: few scattered heterotypic cells were seen in the mucosal tissues. The epithelial glands of the foci were arranged in disorder, and the glandular cavity structure was not obvious. The nuclei were strongly stained and varied in size. Some signet ring-like cells could be seen. Interstitial tissue was connective tissue hyperplasia with chronic inflammatory cell infiltration. (b) PET-CT: the gastric wall of the antrum was slightly thickened with the maximal thickness of 0.7 cm, and abnormal radionuclide activity concentration was found in the corresponding site, SUVmax 3.8, SUVave 2.8. The enlarged lymph nodes could be seen in the right lower abdomen mesentery and the retroperitoneal region of the middle and lower abdomen, the largest was 0.6 × 0.5 cm, and abnormal radionuclide activity concentration was found in the corresponding site, SUVmax 2.5, SUVave 2.4. (c) Pathological report for the specimen of GC distal gastrectomy. The pathological types were signet ring cell carcinoma, poorly differentiated (G3), and invasion to the submucosa. Right: the examination about the lung cancer. (a) The chest PET-CT examination of the patient. A ground glass opacity nodule-like shadow on the left tip of the lung, the size was 1.7 × 1.1 cm, and abnormal radionuclide activity concentration was found in the corresponding site, SUVmax 1.7, SUVave 1.0. (b) Pathological examination of the lung cancer tissue surgical excision. The imaging showed infiltrating lepidic predominant adenocarcinoma. In some regions, the cuboidal cancer cells were growing along the surface of alveolar septa appearing a "hobnail" appearance and part of them infiltrated into the alveoli. The cells had variable size and shape, rich and deeply basophilic cytoplasm, large and dark nucleus, and disorderly arrangement. The fibrous connective tissue hyperplasia accompanied by chronic inflammatory cell infiltration

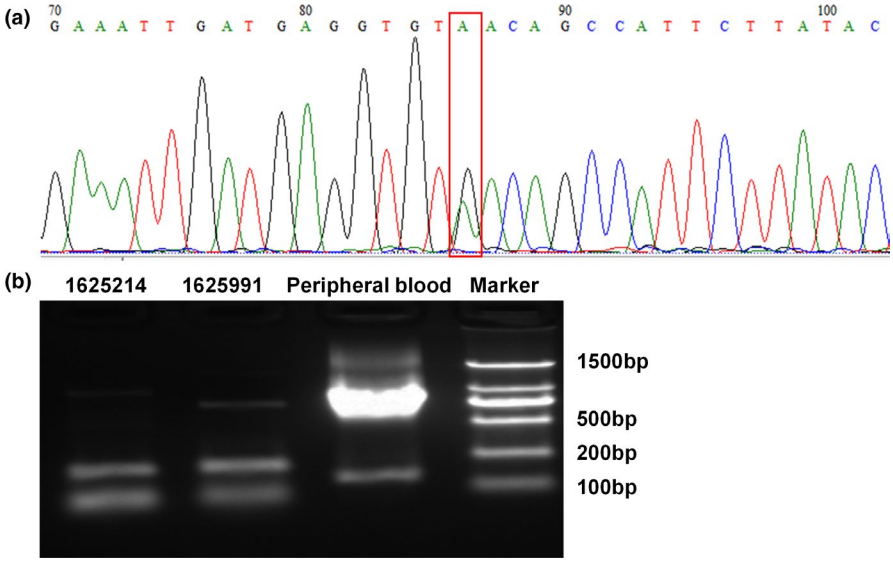

FIGURE 2 Examination and confirmation of the mutL homolog 1 (MLH1) c.1896+5G>A. (a) First-generation sequencing (Sanger) of blood sample of the patient chromatogram result showing G > A at the position. (b) The electrophoretic results of the mRNA MLH1 extracted from tumor specimens and peripheral blood. 1,625,214 was the stomach tumor specimen, 1,625,991 was the lung cancer specimen, peripheral blood is the leucocytes

of the cancer is very high in the same family. This familial onset is mostly autosomal dominant, and called familial cancer syndrome (FCS) or hereditary cancer syndrome (Banks, Moline, Marvin, Newlin, & Vogel, 2013). The disease usually occurs at a younger age (The proband's age of first cancer is usually not more than 50 years old.), and the tumor is not limited to the same organ. In general, if three or more family members (proband and the first and second-degree relatives)

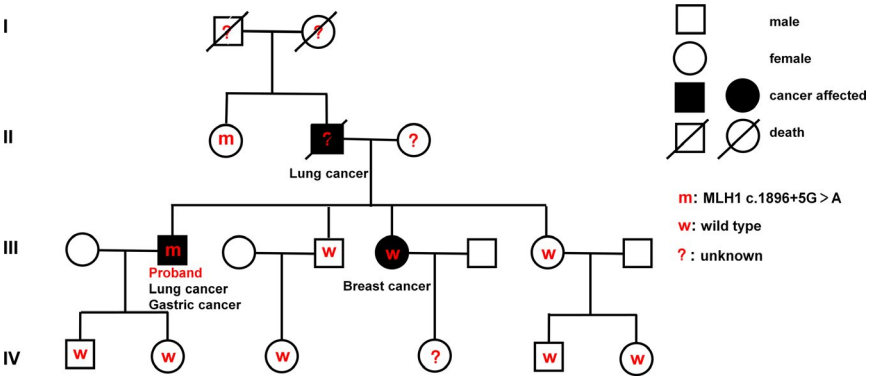

FIGURE 3 Four-generation pedigree of the autosomal dominant inheritance of mutL homolog 1 (MLH1) c.1896+5G>A

have the same type of cancer, it may be considered that the cancer is highly likely to be associated with inheritance (D'Orazio, 2010). Nowadays, with the development of molecular biology and bioinformatics, the NGS detection of specific germline mutations in a family is often used to diagnose and study the FCS (Tafe, 2015). More than 20 types of FCS have been identified so far, such as LS, hereditary medullary thyroid carcinoma (Wells et al., 1982), hereditary breast and ovarian cancer syndrome (Clark & Domchek, 2011) and hereditary diffuse gastric cancer (Grady et al., 2000), etc.

LS is an autosomal dominant FCS characterized by accumulation of CRC in the family. LS was first described by Lynch in 1966 (Lynch, Shaw, Magnuson, Larsen, & Krush, 1966) and named HNPCC in 1985 (Lynch et al., 1985). LS accounts for 2%–5% of all CRC cases. In addition to the significantly higher incidence of CRC, LS patients often develop other cancers and form multiple primary cancers (Hampel et al., 2008). Several gene mutations of the MMR system, including MLH1, MSH2, MSH6, and PMS2, are the main causes of LS. The MMR system guarantees the high fidelity of DNA replication and the MMR inactivation due to gene mutation or promoter methylation leads to the instability of the entire genome and the accumulation of DNA mismatches of many other genes, and then resulting in the carcinogenesis of many tissues, including colorectal, genitourinary, gastric, and hepatobiliary, etc (Larrea et al., 2010).

There are two clinical criteria for diagnosis LS. One is Revised Bethesda Diagnostic criteria: CRC diagnosed at younger than 50 years; presence of synchronous or metachronous CRC or other LS-associated tumors; CRC with microsatellite instability (MSI)-high pathologic-associated features diagnosed in an individual younger than 60 years old; patient with CRC and CRC or LS-associated tumor diagnosed in at least one first-degree relative younger than 50 years old; patient with CRC and CRC or LS-associated tumor at any age in two first-degree or second-degree relatives (Umar et al., 2004). Another one is Amsterdam II criteria: three or more family members (one of whom is a first-degree relative of the other two) with HNPCC-related cancers; two successive affected generations; one or more of the HNPCC-related cancers diagnosed before age 50 years; exclusion of familial adenomatous polyposis (Vasen, Watson, Mecklin, & Lynch, 1999). However, one problem is that the value of current clinical diagnostic criteria is limited in the diagnosis of LS, due to the size of the modern family is getting smaller and smaller and the wide application of colonoscopy has prevented many CRCs. Molecular diagnosis with NGS as the main detection method has made up for the above defects and may even become the most effective and accurate method for the diagnosis of LS. So far, four MMR genes, MLHl, MSH2, MSH6, and PMS2, have been identified that closely related to LS; among them, MLH1 and MSH2 play a major role in LS, and their mutation account for about 90% (MLH1 50% and MSH2 40%) of the cases of LS, and the combined detection rate of MSH6, PMS2, and PMS1 is about 10% (Silva, Valentin, Ferreira Fde, Carraro, & Rossi, 2009). In addition, the penetrance of CRC in LS is not 100% and the range is from 30% to 70% according to different reports (Dunlop et al., 1997; Hendriks et al., 2004), although it is also called HNPCC. Interestingly, the incidence of GC is significantly higher in patients with LS in east Asian populations (Iwama et al., 2004).

4 | CONCLUSION

In this report, the patient's two first-degree relatives had lung cancer and breast cancer, respectively; he had double primary cancers of lung and stomach; MLH1 and MSH2 mutations were detected in GC tissues and lung cancer tissues, respectively. As an East Asian man, GC occurrence was also consistent with previous studies. Therefore, the patient was in line with the diagnostic criteria of LS and we conclude that he had a LS-related carcinoma. The MLH1 c.1896+5G>A detected in lung and gastric tumor specimens was a new germline mutation. The pedigree analysis showed that only the patient and one relative of the previous generation (father's elder sister) carried the mutation, while the sister with breast cancer did not carry the germline mutation. In addition, both the patient and

his father had lung cancer; it seemed that this single nucleotide polymorphism carrying individual might increase susceptibility to some specific kind of cancers, such as lung cancer. Certainly, the clinical significance of The MLH1 c.1896+5G>A germline mutation needs further study.

CONFLICT OF INTEREST

The authors of Qiang Cui and Ming Yao are employees of Origimed, and other authors have no financial conflicts to declare.

AUTHORS CONTRIBUTIONS

XW contributed to conception and the critical revision of the manuscript. XC was in charge of the data interpreting and manuscript writing. XL and LW were responsible for clinical data acquisition and the specimen collection. QC and MY transferred the specimen and performed the gene test and the data analysis. HL mainly reviewed and revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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