



## RAPID COMMUNICATION

# Two novel and one known pathogenic germline mutations in MMRs in Chinese families with Lynch syndrome



Lynch syndrome (LS) is a highly penetrant inherited cancer predisposition syndrome, characterized by autosomal dominant inheritance and germline mutations in DNA mismatch repair (MMR) genes, including *MLH1*, *MSH2*, *MSH6* and *PMS2*. This study aimed to analyze the molecular defects and clinical manifestations of three LS families and propose individual prevention strategies suitable for mutation carriers in different families. The pathogenic gene mutations in each family were identified based on immunohistochemistry results combined with whole-exome sequencing, and the above gene mutations in the other family members were detected using Sanger sequencing. Two novel mutations (c.1927\_1936del, p.I643fs and c.1668-2A > G) in the *MLH1* gene and a known mutation (c.C2228T, p.S743L) in the *MSH2* gene were identified, as pathogenic mutations for LS based on the sequencing data and pattern of tumorigenesis in the family members. The three mutations caused frameshift mutation and abnormal splicing of *MLH1*, as well as base replacement of *MSH2*, resulting in reduced mismatch repair activity of the *MLH1* and *MSH2* proteins. The findings expand the spectrum of known germline mutations of the MMR gene in the Chinese population, and reaffirm the importance of genetic testing for LS.

LS is the most common familial colorectal cancer (CRC) syndrome, accounting for 2–6% of all CRC cases. Pathogenic germline variants in *MLH1*, *MSH2*, *MSH6* and *PMS2* account for 40–60%, 40–50%, 10–20%, and 2% of LS cases, respectively.<sup>1</sup> Patients with LS have a significantly increased risk of developing extra-colonic neoplasia in the endometrium, ovary, pancreas and prostate, etc. They have a lifetime risk of 52–82% for CRC, 40–60% for endometrial cancer, and 6–13% for gastric cancer.<sup>1</sup> Due to a lack of LS-specific clinical symptoms, there is a pressing need to

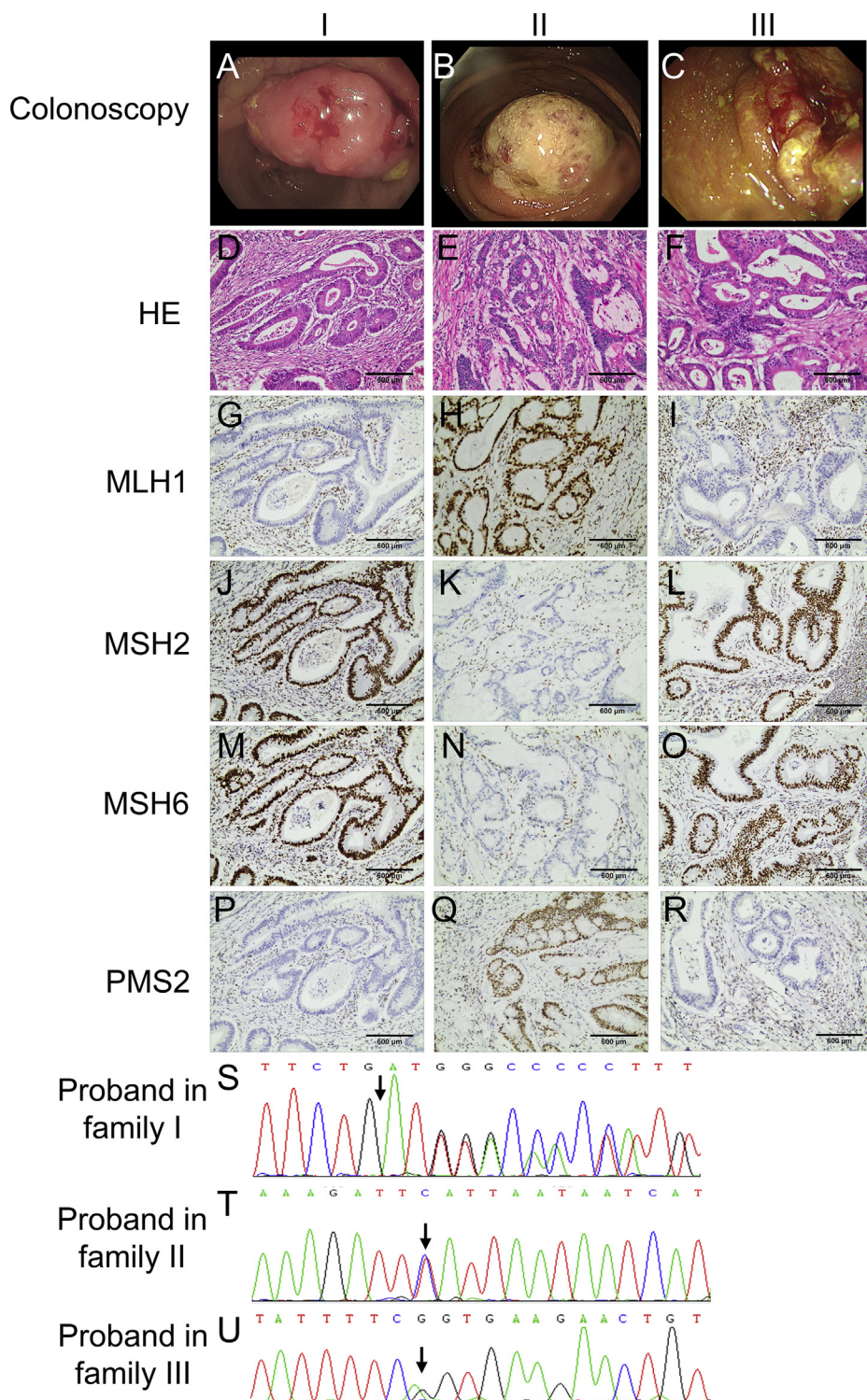
identify consistent molecular markers, correctly identify high-risk individuals and families, as well as implement appropriate management for early diagnosis and prognosis.

We collected clinical data of three LS families from the Central Hospital of Wuhan. The medical and family histories of the three families are shown in [Table S1](#). Methods and Materials can be found in "Supplementary Methods and Materials". The proband of family I was a 62-year-old female who had undergone a partial colon resection due to serrated adenocarcinoma of the right semicolon, her colonoscopy is shown in [Figure 1A](#). Her two younger brothers (II-5, II-6) and daughter (III-4) were diagnosed with colorectal carcinoma and precancerous lesion in the colon, respectively ([Fig. S1](#)). Histopathological examinations on her tumor showed serrated adenocarcinoma ([Fig. 1D](#)) which had infiltrated the entire intestinal wall. No obvious nerve invasion and tumor thrombus were found. Immunohistochemical staining ([Fig. 1G, J, M, P](#)) showed strong positivity for *MSH2* and *MSH6*, weak positivity for *MLH1* and *PMS2*, indicated *MLH1* was the pathogenic gene.<sup>2</sup> Whole-exome sequencing data of the proband is summarized in [Table S2](#). A novel mutation (c.1927\_1936del, p.I643fs) was identified in *MLH1*. This mutation caused a 10-base deletion in exon 17 ([Fig. 1S](#)) of *MLH1* gene, lead to a frameshift mutation starting from isoleucine at 643 and the destruction of the secondary and tertiary structures of *MLH1* protein. The destructed *MLH1* protein was characterized by decreased mismatch repair activity, defective interactions with *PMS2* and *EXO1* (Exonuclease 1), and potential loss of nuclear localization.<sup>3</sup> This mutation was considered to be a pathogenic mutation, and may be inherited from the proband's father. Sanger sequencing was performed to detect this mutation in members of the proband's family in order to provide genetic counseling. The proband's daughter (III-4), who was diagnosed with a precancerous lesion of the colon, was a mutation carrier, but not her son (III-5).

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**Figure 1** Pathological and genetic characteristics in the proband of family. (A) Colonoscopies of the proband in family I: an irregular neoplasm in the ascending colon, with a small amount of dirty moss at the bottom and prone to bleeding (B) Colonoscopies of the proband in family II: a large irregular ring of new growth in the ascending colon. The neoplasm was covered with lichen and prone to bleeding. (C) Colonoscopies of the proband in family III: descending colon (about 30 cm from the anal margin), with a neoplasm (uplifted mass 5 × 4.5 cm), ulceration on the surface, and lumen blockage (D–F) H&E staining of the proband’s tumor tissue in family I, II and III. (G–R) Immunohistochemistry the staining of the proband’s tumor tissue from family I, II, and III, from left to right. The antibodies in each line were specific for MLH1, MSH2, MSH6, and PMS2 in order from top to bottom. Scale bar = 600 μm (S) Sanger sequencing analysis of the proband’s *MLH1* gene (c.1927\_1936del, p.l643fs) in family I: 10 bases were missing in exon 17 (T) Sequencing analysis of the proband’s *MSH2* gene (c.C2228T, p.S743L) in family II: A heterozygous missense mutation in exon 14 was identified. (U) Sequencing analysis of the proband’s *MLH1* gene (c. 1668-2A > G) in family III.

In family II, the proband was a 39-year-old male who was diagnosed with highly-moderately differentiated adenocarcinoma of the ascending colon and had undergone a partial colon resection, his colonoscopy is shown in [Figure 1B](#). His father (II-5) was diagnosed with prostate cancer at the age of 64, and his aunt (II-4) died of lung cancer with brain metastases. His mother and daughter were in good health ([Fig. S2](#)). Histopathological examinations on the tumor showed moderately and poorly differentiated adenocarcinoma, partially exhibited mucinous adenocarcinoma ([Fig. 1E](#)), and the carcinoma had invaded the whole intestinal wall. No nerve invasion but a cancer thrombus was observed. Metastatic carcinoma was discovered in 3/36 periintestinal lymph nodes. Immunohistochemical staining ([Fig. 1H, K, N, Q](#)) showed positivity for MLH1 and PMS2, weak positivity for MSH2 and MSH6, indicated *MSH2* was the pathogenic gene.<sup>2</sup> A known pathogenic heterozygous mutation (c.C2228T, p.S743L, rs63751155)<sup>4</sup> was identified in *MSH2* gene ([Fig. 1T](#)) from the Whole-exome sequencing results ([Table S3](#)). The serine residue at codon 743 of the *MSH2* protein, which was replaced by leucine, is highly conserved, and there is a significant physicochemical difference between serine and leucine. According to algorithms developed to predict the effect of missense changes on protein structure and function (SIFT, PolyPhen-2, Align-GVGD), this variant is likely to be disruptive, but these predictions have yet to be confirmed by published functional studies and their clinical significance is uncertain. The *MSH2* protein is the most robustly expressed protein in primary prostate tumors, and the *MSH2* gene is the most altered MMR gene in advanced prostate cancer. Prostate cancer and LS mediated by the same *MSH2* mutation can coexist in the same family members.<sup>5</sup> Therefore, the father of the proband may have the same genetic mutation which the proband inherited from.

In family III, the proband was diagnosed with adenocarcinoma in the descending colon at the age of 71 and had undergone partial colectomy, her colonoscopy is shown in [Fig. 1C](#). Her older brother and younger sister died of colon cancer and family members III-1 and III-2 were diagnosed with colon cancer ([Fig. S3](#)). Histopathological examinations on her tumor showed moderately and poorly differentiated adenocarcinoma ([Fig. 1F](#)), that had invaded the whole intestinal layer, including surrounding fibrous adipose tissue, with no obvious vascular and nerve invasion in the section. Metastatic carcinoma was discovered in 1/13 periintestinal lymph nodes. Immunohistochemical staining is similar with the proband in family I, also indicated *MLH1* is the pathogenic gene. Whole-exome sequencing results of the proband are shown in [Table S4](#) and we identified another novel mutation of the *MLH1* gene (c.1668-2A > G) ([Fig. 1U](#)). Similar pathogenic mutations of LS in *MLH1* (c.884+4A > G)<sup>2</sup> has previously been reported. This mutation is expected to disrupt mRNA splicing and result in an absent or disrupted protein product, which may induce a decreased mismatch repair activity of the *MLH1* protein.

Sanger sequencing in the proband's family members found no pathogenic mutation carries (III-3, III-4 and III-5).

This study contributes to the genotypic characterization of LS in China, which is important for genetic counseling, diagnosis, cancer prevention, and treatment. Whole-exome sequencing is a quick, accurate, and reliable technique to identify gene variants in suspected LS patients. It has a wide range of potential applications for gene testing of tumors associated with LS. The highest lifetime risks of cancer are attributed to the presence of a mutation in either *MLH1* or *MSH2*. *MSH2* mutation carriers were more prone to developing extracolonic cancers or multiple tumors, while carriers of *MSH6* mutations had a higher possibility of developing endometrial cancer. Therefore, genotype–phenotype correlation studies will allow for more specific treatments for LS patients.

#### Abbreviations

CRC: colorectal cancer; LS: Lynch syndrome; MMR: mismatch repair; MSI: microsatellite instability; PCR: polymerase chain reaction.

#### Author contributions

Conceived and designed the experiments: JY Li, H Huang, KY Huang and AP Deng. Performed the experiments: JY Li, H Huang, KY Huang and AP Deng. Analyzed the data: L Zhu, JY Li and YY Li. Wrote the paper: L Zhu and JY Li. JY Li and L Zhu contributed equally.

#### Conflict of interests

The authors declare that they have no competing interests.

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#### Ethics approval and consent to participate

The study was approved by the Wuhan Central Hospital ethics committee. Informed consent was obtained from the patient's family member.

#### Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.gendis.2021.10.006>.

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