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E-mail: jzus@zju.edu.cn

Correspondence:

A frameshift mutation in exon 19 of *MLH1* in a Chinese Lynch syndrome family: a pedigree study[#]

Qiao-qi SUI[§1,3], Wu JIANG[§1,3], Xiao-dan WU[1,3], Yi-hong LING[2,3], Zhi-zhong PAN[†‡1,3], Pei-rong DING[†‡1,3]

[1]*Department of Colorectal Surgery, Sun Yat-sen University Cancer Center, Guangzhou 510060, China*

[2]*Department of Pathology, Sun Yat-sen University Cancer Center, Guangzhou 510060, China*

[3]*State Key Laboratory of Oncology in South China, Guangzhou 510060, China*

[†]E-mail: panzhzh@sysucc.org.cn; dingpr@sysucc.org.cn

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Lynch syndrome (LS), an autosomal dominantly inherited disease previously known as hereditary non-polyposis colorectal cancer (HNPCC), leads to a high risk of colorectal cancer (CRC) as well as malignancy at certain sites including endometrium, ovary, stomach, and small bowel (Hampel et al., 2008; Lynch et al., 2009). Clinically, LS is considered the most common hereditary CRC-predisposing syndrome, accounting for about 3% of all CRC cases (Popat et al., 2005). LS is associated with mutations of DNA mismatch repair (MMR) genes such as *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM* (Ligtenberg et al., 2009; Lynch et al., 2009), which can trigger a high frequency of replication errors in both microsatellite regions and repetitive sequences in the coding regions of various cancer-related genes. Immunohistochemistry (IHC) tests followed by genetic analysis of these mutations play a significant role in diagnosis, treatment determination, and therapeutic response prediction of LS (Lynch et al., 2009; Alex et al., 2017; Ryan et al., 2017). Here, we report substitution of one base-pair in exon 1 of *MLH3* (c.1397C>A) and a frameshift mutation in exon 19 of *MLH1* (c.2250_2251ins AA) in a 43-year-old Chinese male with an LS pedigree.

The patient was diagnosed with ascending colon cancer, receiving a laparoscopic right hemicolectomy at the age of 43 years in our hospital. His mother was diagnosed with endometrial cancer at the age of 58 years and his maternal grandmother suffered from colorectal cancer and died in her 60 years. His maternal uncle was diagnosed with colon cancer at the age of 50 years. As his family history fulfilled the Amsterdam II criteria for LS, we took a sample of his tumor for IHC analysis, which revealed defects in MLH1 and PMS2 proteins (Figs. 1a and 1b).

We then undertook genetic analysis of genes related to hereditary CRC to further characterize the mutations. Next generation sequencing was performed by polymerase chain reaction (PCR)-direct sequencing analysis, targeting genes including *APC*, *AXIN2*, *EPCAM*, *MLH1*, *MLH3*, *MSH2*, *MSH6*, *MUTYH*, *PMS1*, *PMS2*, *STK11*, *PTEN*, *SMAD4*, and *BMPR1*. Results were compared with the "1000 Genomes" browser (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes) and the pathogenicity of mutations was classified according to the recommendations of the American College of Medical Genetics and Genomics (ACMG; http://www.acmg.net). After excluding promoter methylation, the analysis showed a heterozygous substitution of one base-pair (c.1397C>A) in exon 1 of *MLH3*, whose transcript variant (p.Ser466Ter) leads to a protein truncated at codon 465 instead of codon 1429 in the wild type. Also, a heterozygous frameshift mutation in exon 19

‡ Corresponding authors
§ The two authors contributed equally to this work
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ORCID: Qiao-qi SUI, https://orcid.org/0000-0001-5191-218X

of *MLH1* (c.2250_2251insAA) was detected, which created a new reading frame at Val 752, encountering a premature stop codon at the following 32nd position (p.Val752L-ysfs*32) and eventually a protein of 782 codons instead of 757 in the wild type (Fig. S1). Both mutations were found to be dominant. Comparisons with previous findings from the "1000 Genomes" browser and ACMG recommendations showed that the mutation in *MLH3* had not been previously reported. Therefore, we considered it to be a suspected pathogenic mutation. Although not previously reported as pathogenic, similar *MLH1* nucleotides, detected in a South American LS pedigree (c.2252_2253dup-AA) (Dominguez-Valentin et al., 2013) and a Chinese pedigree (Sheng et al., 2008), were recognized as variants of unclassified significance (VUS).

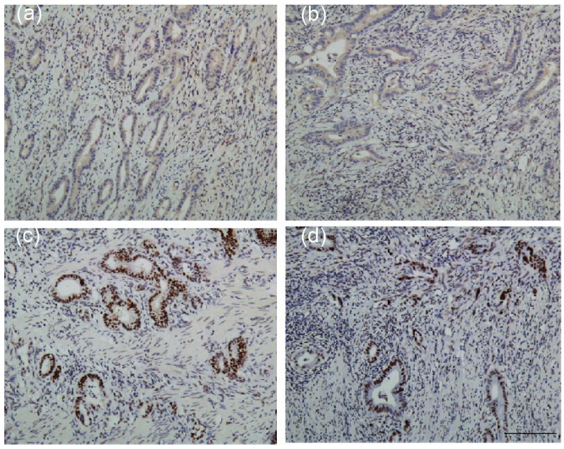

Fig. 1 IHC examination of MMR protein expression in tumor sections

IHC examination revealed loss of MLH1 (a) and PMS2 (b) expression in tumor cells, while no loss of MSH2 (c) or MSH6 (d) expression was observed. Scale bar=200 μm. IHC: immunohistochemistry; MMR: mismatch repair

To confirm the germline mutation in those genes as well as the pathogenic effect of the *MLH3* variants, we provided genetic counseling for the patient's family members via Sanger sequencing, and recommended genetic analysis. The analysis showed that the germline mutation in *MLH1* was carried by the patient's brother, niece, and mother (Fig. S2a), while the same *MLH3* variant was detected only in the patient's father who was never diagnosed with a tumor (Fig. S2b). These results suggested that the mutation in *MLH1* weighed more in the LS etiology of the proband. Genetic analysis of the maternal uncle with colon cancer was not carried out due to personal refusal. The pedigree chart is shown in Fig. 2.

Genetic linkage analysis showed that 80% of LS cases are related to germline mutations in *MSH2* and *MLH1* (Alex et al., 2017), whereas the mutation we found in *MLH1*, previously reported in only one pedigree of a South American, was predicted to be a VUS by multiple in silico tools (Dominguez-Valentin et al., 2013). Germline mutation was revealed in the maternal pedigree, especially in patients with LS involving malignancy, which supports its etiological role according to the law of segregation. Also, deficiencies in both MLH1 and PMS2 were proved by IHC tests in the proband. We believe that an abnormal C-terminus in the MLH1 protein caused by such a

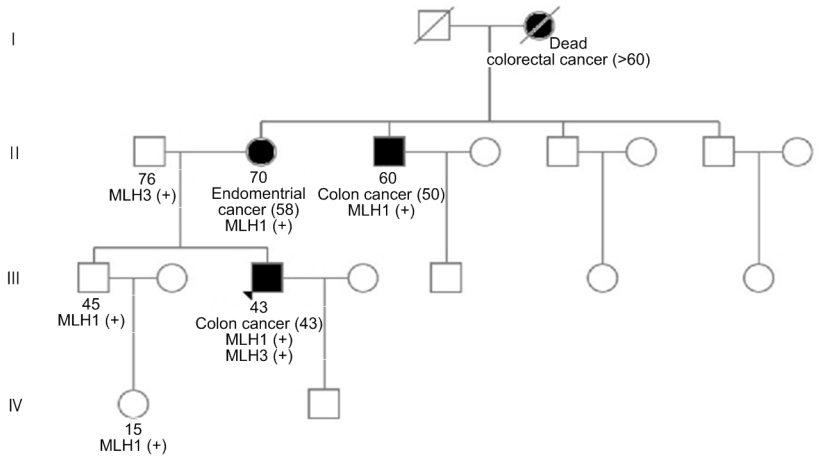

Fig. 2 Pedigree chart of the family with Lynch syndrome

Black symbols indicate individuals diagnosed with cancers, with slashes indicating death. Ages upward mean the current age (years), while ages in the brackets indicate diagnosed age (years). A plus symbol indicates the mutation in the mismatch repair (MMR) genes. The triangle indicates the proband. Square: male; Circle: female

mutation could provide an explanation. It was reported that the heterodimer of MLH1 combined with PMS2 serves as a major effector of MMR function in vivo, and the participation of the carboxy terminal homology (CTH) domain (residues 500–756 amino acids in MLH1) in the structure is crucial for its stabilization (Mohd et al., 2006). Moreover, mutations truncating or expanding MLH1 peptide are related to the loss of such domains (Kim et al., 2009), theoretically triggering the absence of MLH1 and PMS2. For these reasons, the *MLH1* variant carried by the proband, extending the peptide and affecting the structure of the CTH domain, was supposed to destroy the stability of the heterodimer, causing MMR deficiency and eventually LS. Nevertheless, previous studies reported that patients with a defective MMR had a mean age of 45 years (Lynch et al., 2009), while patients in our study carrying only the *MLH1* mutation were diagnosed after their 50 years. Further studies concerning the significance of similar variants are required.

We had suspected that the mutation in *MLH3* plays an important role in tumorigenicity, but the results from genetic analysis suggested the contrary. The father lived tumor-free as a carrier of *MLH3* mutation alone, which did not support individual pathogenicity. Clinically, there have been few reports concerning tumor predisposition with only *MLH3* mutations (Hienonen et al., 2003; Mohd et al., 2006), providing little evidence. Also, Korhonen et al. (2008) functionally characterized seven missense mutations of *MLH3*, and disproved their individual function of interfering with MMR, describing them as low-risk mutations for LS.

Although the mutation in *MLH1* was considered pathogenic in our case, we still believed that *MLH3* variant made a difference. The proband was diagnosed with colon cancer at the age of 43 years, much younger than the other patients in his family. In addition, malignancy has not yet been diagnosed in his 45-year-old brother carrying *MLH1* variant. Therefore, it is possible that *MLH3* deficiency enhanced the tumorigenicity of *MLH1* mutation and accelerated the occurrence of the tumor. According to the study of Chen et al. (2005), *MLH3*, following *PMS2*, takes part in tumor suppression in mice with *MLH1*. Also, Chen et al. (2008) showed that a loss of *MLH3* accelerates the progression of gastrointestinal tumors on the basis of *PMS2* deficiency. We believe that the compound heterozygote of these germline mutations is more likely to trigger LS. Further studies are needed to provide more conclusive evidence, as this is the first time that this phenomenon has been detected.

In conclusion, we identified a mutation in exon 19 of *MLH1* and a novel germline mutation in exon 1 of *MLH3*. The mutation in *MLH1*, although described previously as a VUS, was detected in the maternal pedigree and possibly resulted in LS. *MLH3* variant was not detected among other patients of the maternal pedigree, but we still believe it may have enhanced tumorigenicity in this case.

Contributors

Qiao-qi SUI and Wu JIANG wrote this report and collected references. Xiao-dan WU drew the figure of genetic reports and pedigree chart. Yi-hong LING diagnosed defective MMR and drew the figure of IHC examination. Pei-rong DING wrote an outline. Zhi-zhong PAN checked and approved the final version.

Compliance with ethics guidelines

Qiao-qi SUI, Wu JIANG, Xiao-dan WU, Yi-hong LING, Zhi-zhong PAN, and Pei-rong DING declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study. Additional informed consent was obtained from all patients for whom identifying information is included in this article.

References

Alex AK, Siqueira S, Coudry R, et al., 2017. Response to chemotherapy and prognosis in metastatic colorectal cancer with DNA deficient mismatch repair. *Clin Colorectal Cancer*, 16(3):228-239. https://doi.org/10.1016/j.clcc.2016.11.001

Chen PC, Dudley S, Hagen W, et al., 2005. Contributions by mutl homologues *Mlh3* and *Pms2* to DNA mismatch repair and tumor suppression in the mouse. *Cancer Res*, 65(19):8662-8670. https://doi.org/10.1158/0008-5472.CAN-05-0742

Chen PC, Kuraguchi M, Velasquez J, et al., 2008. Novel roles for MLH3 deficiency and TLE6-like amplification in DNA mismatch repair-deficient gastrointestinal tumorigenesis and progression. *PLoS Genet*, 4(6):e1000092. https://doi.org/10.1371/journal.pgen.1000092

Dominguez-Valentin M, Nilbert M, Wernhoff P, et al., 2013. Mutation spectrum in south american lynch syndrome

families. *Hered Cancer Clin Pract*, 11(1):18. https://doi.org/10.1186/1897-4287-11-18

Hampel H, Frankel WL, Martin E, et al., 2008. Feasibility of screening for lynch syndrome among patients with colorectal cancer. *J Clin Oncol*, 26(35):5783-5788. https://doi.org/10.1200/JCO.2008.17.5950

Hienonen T, Laiho P, Salovaara R, et al., 2003. Little evidence for involvement of *MLH3* in colorectal cancer predisposition. *Int J Cancer*, 106(2):292-296. https://doi.org/10.1002/ijc.11218

Kim KH, Kim JY, Oh SI, et al., 2009. A novel germline mutation of *hMLH1* in a korean hereditary non-polyposis colorectal cancer family. *Int J Oncol*, 34(5):1313-1318. https://doi.org/10.3892/ijo_00000258

Korhonen MK, Vuorenmaa E, Nyström M, 2008. The first functional study of *MLH3* mutations found in cancer patients. *Genes Chromosomes Cancer*, 47(9):803-809. https://doi.org/10.1002/gcc.20581

Ligtenberg MJL, Kuiper RP, Chan TL, et al., 2009. Heritable somatic methylation and inactivation of *MSH2* in families with Lynch syndrome due to deletion of the 3' exons of *TACSTD1*. *Nat Genet*, 41(1):112-117. https://doi.org/10.1038/ng.283

Lynch HT, Lynch PM, Lanspa SJ, et al., 2009. Review of the lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications. *Clin Genet*, 76(1):1-18. https://doi.org/10.1111/j.1399-0004.2009.01230.x

Mohd AB, Palama B, Nelson SE, et al., 2006. Truncation of the C-terminus of human MLH1 blocks intracellular stabilization of PMS2 and disrupts DNA mismatch repair. *DNA Repair*, 5(3):347-361. https://doi.org/10.1016/j.dnarep.2005.11.001

Popat S, Hubner R, Houlston RS, 2005. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol*, 23(3):609-618. https://doi.org/10.1200/JCO.2005.01.086

Ryan E, Sheahan K, Creavin B, et al., 2017. The current value of determining the mismatch repair status of colorectal cancer: a rationale for routine testing. *Crit Rev Oncol Hematol*, 116:38-57. https://doi.org/10.1016/j.critrevonc.2017.05.006

Sheng JQ, Fu L, Sun ZQ, et al., 2008. Mismatch repair gene mutations in Chinese HNPCC patients. *Cytogenet Genome Res*, 122(1):22-27. https://doi.org/10.1159/000151312

List of electronic supplementary materials

Fig. S1 Sequencing of the proband

Fig. S2 Sequencing of the patients of the Lynch syndrome pedigree

中文概要

题　目：一个中国 Lynch 综合征家系携带的 *MLH1* 基因第 19 号外显子移码突变：一项家系研究

目　的：寻找一个 Lynch 综合征患者所在家系携带的 DNA 错配修复基因突变，探讨各突变对肿瘤发生发展的影响。

创新点：*MLH1* 的第 19 号外显子 c.2250_2251insAA 移码突变既往被认为是意义未名突变，而我们的研究为明确该突变的致病意义提供了依据。另外，我们首次报道了 *MLH3* 基因第 1 号外显子 c.1397C>A 突变。该突变有可能使 Lynch 综合征患者的发病年龄提前。

方　法：运用免疫组织化学技术检测家系先证者肿瘤组织中错配修复基因蛋白的缺失情况，使用二代测序技术通过先证者血标本明确患者所携带的突变。同时运用 Sanger 法检测家系其他成员该突变的携带情况以明确突变对肿瘤发生发展的影响。

结　论：我们在患者体内发现 *MLH1* 基因第 19 号外显子移码突变（c.2250_2251insAA）以及 *MLH3* 基因第 1 号外显子 c.1397C>A 突变。在患者家系中，我们仅检测到有 *MLH1* 突变，因此该突变极有可能为致病突变。

关键词：Lync 综合征；家系；DNA 错配修复基因；置换；移码突变