

A Hereditary Mutation of MSH2 Gene Associated with Lynch Syndrome in a Five Generation Chinese Family

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Purpose: In order to clarify which variants of the MMR gene could provide current “healthy” members in affected families a more accurate risk assessment or predictive testing.

Patients and Methods: One family, which meets the criteria according to both Amsterdam I/II and Bethesda guidelines, is reported in this study. The proband and some relatives of the patient have been investigated for whole genome sequencing, microsatellite instability, immunohistochemical MMR protein staining and verified by Sanger sequencing.

Results: A heterozygous insertion of uncertain significance (c.420dup, p.Met141Tyrfs) in MSH2 gene was found in proband (III-16) and part of His relatives. The variant was associated with a lack of expression of MSH2 protein (MMR deficient) and high microsatellite instability analysis (MSI) status in tumor tissues of LS patients. In addition, we found that the variant could affect the expression of MSH2 and the response to chemotherapy drugs in vitro.

Conclusion: We identified an insertion mutation (rs1114167810, c.420dup, p.Met141Tyrfs) in MSH2 in LS using whole genome-wide sequencing (WGS). We further confirmed that this mutation plays an important role in LS patients of this pedigree based on in vivo and vitro study.

Keywords: Lynch syndrome, genetic variation, mismatch repair gene, MSH2, chemotherapy resistance

Introduction

Colorectal cancer is one of the largest public health problems in the world. It is clear that 20% to 30% of colorectal cancers (CRCs) are hereditary, with 5% to 10% related to a known genetic syndrome such as Lynch syndrome (LS).^{1,2} LS is an autosomal dominant syndrome occurring cancers in colorectum, endometrium, stomach, small bowel, ovarian, ureter, renal pelvis, brain, hepatobiliary tract and sebaceous gland.³⁻⁶ Available data show that LS accounts for 1% to 3% of all CRC, and LS patients may suffer from early cancer development and increased risk of extra-colonial manifestations.⁷⁻¹¹ The mean age of LS onset is 44–61 years, which is younger than that of sporadic CRC patients.¹²⁻¹⁵ Besides, colorectal cancer risks are reported to be as high as 75% in LS.¹⁶ Furthermore, the average time from onset of a polyp to the onset of carcinoma is much shorter in LS (2–3 years) compared with sporadic colorectal cancer (4–10 years).^{17,18}

The etiology of LS is a deficiency of mismatch repair (MMR) system that responsible for the surveillance and correction of errors in DNA during replication,

repair and recombination.^{19,20} Once pathogenic mutations are found to contribute to MMR function deficiency clearly, mutation carriers can be benefited from genetic counseling. Thus, screening pathogenic mutations in MMR genes is critical for diagnosis, monitoring and management of LS.

MutS homologue 2 (*MSH2*), mutL homologue (*MLH1*), mutS homologue 6 (*MSH6*), post-meiotic segregation increased 2 (*PMS2*) and epithelial cell adhesion molecule (*EPCAM*) are found to play important roles in MMR.^{21–24} Germline mutations in *MLH1*, *MSH2*, *MSH6*, *PMS2* and *EPCAM* genes accounts for approximately 50%, 40%, 7–10%, less than 5% and 3% cases of LS.^{25–28} LS patients with mutations in these genes have a higher rate of spontaneous somatic mutations in microsatellite sequences that result in microsatellite instability (MSI).²⁹ Both large rearrangements and single nucleotide variant (SNV) in these genes may alter MMR protein function.^{22,30} Insertion, deletion or nonsense SNVs can cause truncated proteins, while exonic or intronic variants located in splicing sites can result in aberrantly spliced mRNA transcripts.^{31,32} Since there are many mutations in MMR genes, it is important to identify the potential causative genetic variants of LS.

MSH2 was the first identified LS causative gene.³³ It interacts with *MSH6* or *MSH3* to form the MutS α / β complexes, and they translocate into the nucleus to bind with DNA in order to initiate the repair process.³³ Many evidences indicated that *MSH2* plays a vital role in the MMR system, and numerous germline mutations of *MSH2* are discovered.³⁴ At present, 119 variants of *MSH2* have been reported in the Chinese population, including 51 substitutions, 25 frameshifts, 10 nonsenses, 27 deletions, 9 insertions and 4 duplications.³⁵ However, how many variations of them are causative genetic variants of LS remains unknown. In addition, it is unclear if there are unknown mutations that contribute to LS. Anyway, clarifying the pathogenic role of genetic mutations in *MSH2* may contribute to diagnose, monitor and manage LS.

In this study, we identified an insertion mutation (rs1114167810, c.420dup, p.Met141Tyrfs) in *MSH2* which has not been reported in LS using whole genome-wide sequencing (WGS). We further confirmed that this mutation plays an important role in LS patients of this pedigree based on in vivo and vitro study.

Methods and Materials

Patients and Samples

Samples (including 102 whole blood samples and 3 colorectal cancer tissues) of patients and the family, as well as basic information, were collected from Xiangya Hospital, Central South University (Changsha, Hunan, China) from 2015 to 2018. The purpose of this study was informed to all patients or their relatives, and consent forms were signed by them. The study protocol was approved by the Ethics Committee of Xiangya School of Medicine, Central South University.

DNA Extraction

Germline DNA for whole genome sequencing and Sanger sequencing was extracted from the whole blood of the participants using the QIAamp DNA Blood Midi Kit (Qiagen; Valencia, CA, USA). Formalin-fixed paraffin-embedded (FFPE) DNA for microsatellite instability (MSI) analysis was extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen; Valencia, CA, USA).

Whole Genome Sequencing

Firstly, DNA was sheared using Covaris focused-ultrasonication (Covaris, MA, USA), and DNA fragments were enriched by 6 cycles of PCR. Then, libraries were analyzed for size distribution by Agilent 2100 Bioanalyzer. Finally, the DNA libraries were sequenced on Illumina Hiseq platform and 150-bp paired-end reads were generated as raw reads.

Data Processing

Raw reads in FastQ format that contained adapter contamination and low-quality/unrecognizable nucleotides were discarded using Trimmomatic (0.36 version). Reads after quality control were aligned to the UCSC human reference genome (GRCh37) using BWA.³⁷ Samtools and Picard were used for sorting, removing PCR duplicates, and building an index for the bam files. Then, base quality recalibration was performed by GATK to generate final BAM files for mutation calling.³⁸ Germline single nucleotide variations (SNVs) and small insertions and deletions (InDels) were called by GATK, the resulting variants were annotated and prioritized by ANNOVAR.³⁹ The pathogenicity of missense variants was evaluated by SIFT, PolyPhen2, and MutationTaster. We focused on pathogenic and expected pathogenic mutations in several genes (including: *MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*, *APC*, *MUTYH*, *PTEN*, *STK11*, *TP53*, *SMAD4* and *BMPRIA*) that were defined to be high-risk genes in CRC by the American College of Medical Genetics. Only coding and

splice region variants were considered and all variants identified in this study were manually inspected using Integrative Genomics Viewer (IGV version 2.3.86).⁴⁰ Control-FREEC and Breakdancer were utilized to detect copy number variations (CNVs) and structural variations, respectively.^{41,42} All these variant results were visualized using Circos.⁴³

Sanger Sequencing

Sanger sequencing was used to validate the candidate variants identified above. PCR primers were designed with Primers5 tools. PCR amplification was carried out in ABI 9700 Thermal Cycler. Sequence data comparisons and analysis were performed by SeqMan (DNASTAR, Madison, Wisconsin, USA). Sequences of primers for identifying these candidate loci are given in [Table S1](#).

Microsatellite Instability Analysis

MSI was performed for the proband's mother (II-3) with genomic DNA which was isolated from tumor samples and corresponding blood, respectively. A 5-marker panel including two mononucleotide repeats (BAT25 and BAT26) and three dinucleotide repeats (D2S123, D5S346 and D17S250) which recommended by the National Cancer Institute Workshop on MSI for Cancer Detection and Familial Predisposition was used.⁴⁴ Oligonucleotide primers were fluorescently labelled and PCR products were evaluated using 3500DX Genetic Analyzer. Tumors were classified as highly unstable (MSI-H) if at least 40% of the markers showed instability.

Immunohistochemistry

All tissue specimens were collected via biopsy of paraffin-embedded samples for immunohistochemistry (IHC) analysis in the Pathology Department of Xiangya Hospital or Hunan Provincial Tumor Hospital. Tissue sections (4 μm thick) were cut from paraffin-embedded blocks. Antibodies used were: Anti-Human MutL Protein Homolog 1, Clone ES05 (DAKO ref:IR079), Anti-Human Postmeiotic Segregation, Clone EP51 (DAKO ref: IR087), Anti-Human MutS Protein Homolog 6, Clone EP49 (DAKO ref: IR086).

Plasmid Construction

We constructed both wild-type and c.420dup mutant MSH2 expression plasmids in this study. To get the wild-type MSH2 plasmid, full-length MSH2 (NM_000251.2) coding regions were cloned into the pCDNA3.1 vector

(Invitrogen, CA, USA) at the multiple cloning site of BamHI and EcoRI. To generate the mutant (c.420dup; p. Met141Tyrfs) MSH2 plasmids, site-directed mutagenesis was carried out. All constructs were directly sequenced to confirm the inserted fragments. Primers used in plasmid construction were also presented in [Table S1](#).

Cell Culture and Transfection

Human colon cancer cell HCT116 cells were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). These cells were cultured in RPMI-1640 media, supplemented with 12% fetal bovine serum (FBS) at 37°C under an atmosphere of 95% air and 5% CO₂. The cells for transfection were cultured on six-well plates and grown till 70–90% confluent. Wild-type and c.420dup mutant MSH2 constructs were separately transfected into the cells in comparable amounts using Lipofectamine 3000 (Invitrogen Corporation, Carlsbad, CA, USA).

Western Blot

Twenty-four hours after transfection, the cultured HCT116 cells were harvested and analyzed by Western blotting with an antibody to MSH2. Proteins were extracted using RIPA buffer mixed with protease inhibitors (1:100), phenyl methyl sulfonyl fluoride (PMSF, 1:100) and dithiothreitol (DTT, 1:100) at 4°C for 30-mins incubation. The lysate was centrifuged at 13,000 rpm at 4°C for 15 min. The supernatants were collected and protein concentrations were measured using the BCA method. Forty micrograms of extracts were denatured in SDS loading buffer (TaKaRa), loaded on the gel, separated by 12% SDS-PAGE gel electrophoresis and transferred to PVDF membranes (Millipore, Massachusetts, USA). The membranes were blocked using 5% skim milk and incubated at 4°C overnight with the following primary antibodies: anti-MSH2 antibody (1:1000, CST, China) and anti-β-catenin (1:1000, CST, China), followed by anti-rabbit IgG (CST, China) and anti-mouse IgG (CST, China) at a 1:3000 dilution for 2 hrs at room temperature. Enhanced chemiluminescence (ECL) reagents (Super Signal West Femto Maximum Sensitivity Substrate, Thermo Fisher Scientific, Rockford, USA) were used and the signal was detected with Bio-Rad ChemiDoc XRS. The intensity of them was normalized relative to β-actin bands and was analyzed by ImageJ software.

The expression level of MSH2 was calculated as the mean of three independent experiments.

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

We extracted total RNA from tissue specimens using Trizol reagent (Takara) according to the manufacturer's protocol. Nano Drop Spectrophotometer (Shimadzu Biotech, Beijing China) and gel electrophoresis were employed to measure the quality and quantity of extracted RNA. The isolated RNA concentration was calculated and normalized with RNase-free water and then reverse-transcribed into cDNA using PrimeScript™ RT reagent kit with gDNA Eraser (RR047A; Takara, Dalian, China). All cDNA samples were stored at -80°C until use. Light Cycle@480 II (Roche, Basel, Switzerland) was employed to conduct qRT-PCR by using TB Green™ Premix Ex Taq™ II (Tli RNaseH Plus, Code No: RR820A, Takara Bio Inc.). The qRT-PCR amplification was performed as follows: an initial denaturation at 95°C for 30 s, followed by 40 cycles at 95°C for 5 s, 60°C for 30 s. The $2^{-\Delta\Delta\text{Ct}}$ was used to quantify the fold change of MSH2 expression in HCT116 cell. The relative expressions of target genes were calculated after normalization against a reference

gene (GAPDH). All primers used for qPCR were listed in Tables.

Cell Viability Analysis

Oxaliplatin, 5-fluorouracil and Irinotecan were purchased from Dalian Meilun Biology Technology Co., Ltd. (Dalian, P.R. China) and was diluted with DMSO (Sigma, St Louis, MO) and stored at the concentration of 100 mM. Cell viability was detected by the MTS approach according to the protocol for Cell Titer 96 Aqueous-One-Solution Cell Proliferation Assay kit (MTS). HCT116 cells transfected with wild and mutation *MSH2* were seeded in 96-well plates at a density of 5×10^3 cells in 100- μL medium. Plated cells were incubated with Oxaliplatin, 5-fluorouracil and Irinotecan (CST) for 48 hrs respectively. The medium was then replaced by 100- μL MTS and RPMI-1640 media with a ratio of 1:9. Cell viability was then obtained by measuring the absorbance at a wavelength of 450 nm. All experiments were performed at least three times. The IC50 was calculated and the dose-response curves were depicted using GraphPad Prism 6.0 program.

Statistical Analysis

Statistical analysis was performed with SPSS 16.0 (SPSS Inc, Chicago, IL, USA). Results expressed as mean \pm SD

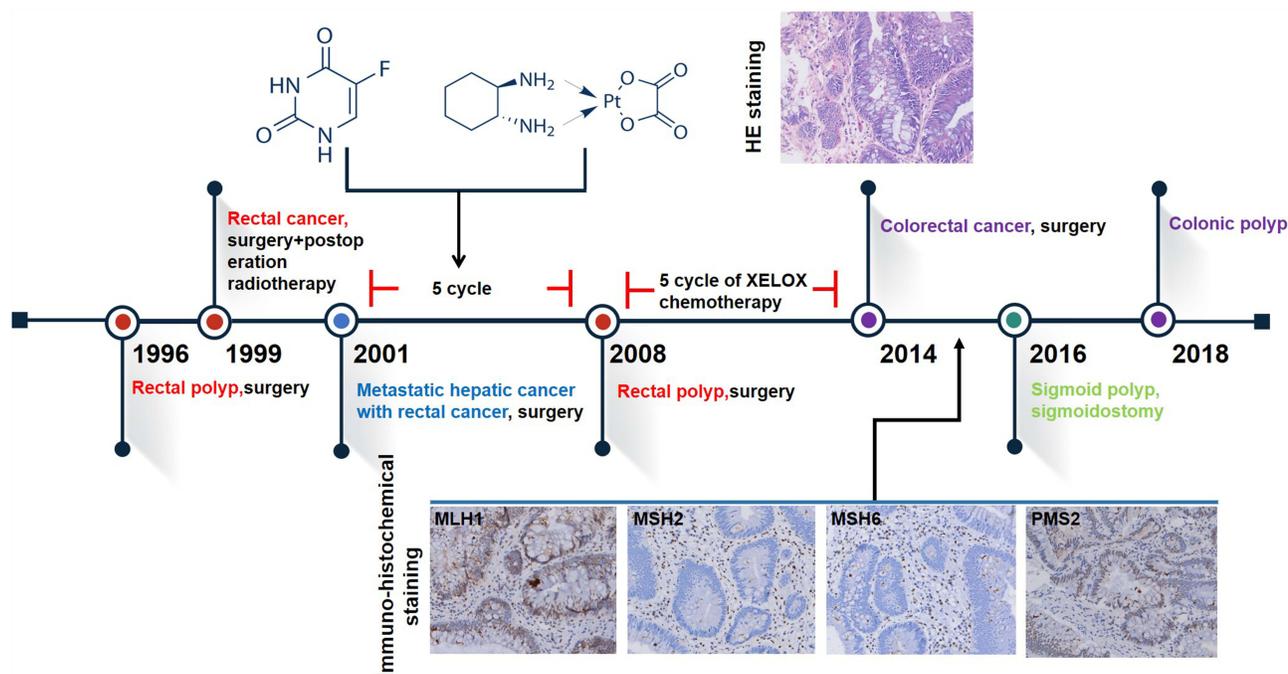


Figure 1 Clinical diagnosis and treatment process of the proband (III-16). HE staining of colon tissue from proband showing a colon adenocarcinoma. Immunohistochemical staining results for adenocarcinoma in proband. There is a loss of expression of MSH2 and MSH6 in the neoplastic cells. The tumor cells retain the nuclear expression of MLH1 and PMS2.

were analyzed using the Student's *t* test. Differences were considered significant when $P < 0.05$.

Information Collection of MSH2

The information on annotated domains and conservations of *MSH2* is collected in *Pfam* (version 32.0, <http://pfam.xfam.org/protein/P43246>). The structure prediction of *MSH2* is performed by *Phyre2* (version V 2.0, <http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index>).

Results

A 5-Generation LS Pedigree Was Found

The proband (III-16) was a 48-year-old male, whose personal and family history meet Amsterdam II criteria for LS. He was diagnosed with rectal cancer at the age of 28, and then received radical resection of rectal carcinoma and post-operation radiotherapy. At the age of 33, he developed metastatic hepatic cancer and then underwent right hemihepatectomy and 5 cycles of chemotherapy. When he was 40, he was diagnosed with rectal cancer and received both surgery and 5 cycles of chemotherapy. He developed colon cancer and subsequently received transverse colonic ostomy and partial small intestine resection when he was 46. Later, he developed sigmoid polyps and rectal polyps at the age of 48 and 49, respectively. The whole process of diagnosis and therapy of this patient is summarized in Figure 1.

Then, we investigated the five generations of the proband's family and found proband's family showed a typical LS pedigree based on Amsterdam II criteria (Figure 2). Among 102 members, five of them (II-3, III-5, III-20, III-26 and IV3) were diagnosed as colorectal cancer. III-24 and III-22 were with stomach cancer and

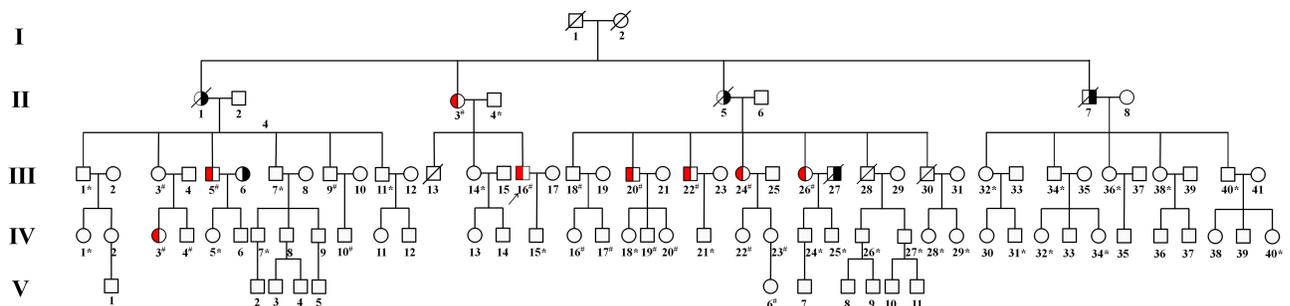
colon adenoma, respectively. Another three family members (II-1, II-5 and II-7) had already died of various cancer. II-7 was diagnosed with nasopharyngeal carcinoma at 50 and died at 56. II-5 was diagnosed with brain stem glioma at the age of 50, and II-1 developed Kidney cancer. Detailed patient family history was collected and summarized in Table 1.

MSI Was Found in the Pedigree

Since MSI is a symbol of deficiency MMR function, we then performed the MSI test in this pedigree. Tumors from a member who was diagnosed as colorectal cancer (II-3) were collected and used for the MSI test. The 5-marker panel including two mononucleotide repeats (BAT25 and BAT26) and three dinucleotide repeats (D2S123, D5S346 and D17S250) were used. BAT25 and D2S123 were MSS, while BAT26, D17S250 and D5S346 were MSI (Figure 3A). These results indicated that II-3 was confirmed to MSI-high state because three of five tested loci were found altered (60% MSI).

The Deficiency of MSH2 and MSH6 Were Found in the Pedigree

MSI is often correlated with defect MMR function deficiency, we then detected the expression of MMR proteins (MLH1, MSH2, MSH6 and PMS2) directly to make sure if MMR deficiency exists in this pedigree. Immuno-histochemical staining ($\times 20$) of tumor tissues from two colorectal cancer members (II-3 and III-16) both demonstrate that MSH2 and MSH6 are absent in the tumor cell nuclei while they are presented in the



Graphic symbol

□ male ○ female ● Lynch syndrome ● NA

Figure 2 Pedigree of the Chinese family with LS. The arrow indicates the proband (III-16). Squares and circles denoted males and females, respectively. Red filled symbols indicate LS members, black filled symbols indicate those members with tumors cannot be diagnosed as Lynch syndrome, and empty symbols indicate unaffected individuals. Sign “#” indicates that family members were tested for mutations and found to carry the mutation in the pedigree; Sign “*” indicates family members who were tested and found not to carry the mutation.

Table 1 Clinical Characteristics of Patients in LS Family

Subjects	Sex	Status	Ave	Age at Onset	Tumor Type	Pathologic Types	Pathological Stage	Treatment	MSI	Microscopy	IHC	Lynch Syndrome
III-16	M	Alive	48	26	CRC	AC	B2	SU+CH	MSI-H	Y	-- ++	Y
II-3	F	Alive	67	64	CRC	AC	B2	SU	MSI-H	Y	-- ++	Y
III-5	M	Alive	50	50	CRC	AC	C	SU	NA	Y	NA	Y
IV-4	F	Alive	29	21	CRC	AC	B2	SU+CH	NA	Y	NA	Y
III-26	F	Alive	61	43	CRC	AC	B1	SU	NA	Y	NA	Y
III-24	F	Alive	53	45	SC	AC	C	SU+CH	NA	Y	NA	Y
III-20	M	Alive	45	43	CRC	AC	B0	NA	NA	Y	NA	Y
III-22	M	Alive	48	47	CA	AD	NA	SU	NA	Y	NA	Y
III-27	M	Died	57	NA	SC	NA	NA	CH	NA	N	NA	N
II-1	F	Died	88	NA	RC	NA	NA	NA	NA	N	NA	NA
II-5	F	Died	50	NA	BG	NA	NA	NA	NA	N	NA	NA
I-7	M	Died	56	51	NC	NA	NA	NA	NA	N	NA	NA

Abbreviations: M, male; F, female; Y, yes; N, no; NA, not available; MSI, microsatellite instability; IHC, immunohistochemistry; CRC, colorectal cancer; SC, stomach cancer; IV, colonic adenoma; RC, renal cancer; BG, brainstem glioma; NC, nasopharyngeal carcinoma; AC, adenocarcinoma, AD, adenoma; SU, surgery; CH, chemotherapy; IHC-4, immunohistochemistry (MSH2, MSH6, MLH1, PMS2).

normal internal control (non-tumor cells) (Figure 3B). The II-3 is the mother of III-16 so that they may suffer from a heritable factor. These results are summarized in Table 2.

A Frameshift INDEL Mutation in *MSH2* Was Found in the Pedigree

To explore the genetic factor for LS, whole-genome sequencing was performed on the proband (III-16). A total of 4023,831 germline SNVs and 852,009 germline InDels were called. All variations, including SNVs, InDels, copy number variations and structural variations are shown in Figure 4A. The distribution of SNVs and Indels in genome regions and the type of mutation in the coding region of exon are showed in Figure 4B. SNVs and InDels which were identified or expected as pathogenic mutations in CRC by the American College of Medical Genetics in several genes including: *MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*, *APC*, *MUTYH*, *PTEN*, *STK11*, *TP53*, *SMAD4* and *BMPRIA*. High frequency of variation was found in high-risk genes of Lynch syndrome. And we found that seven mutations which locate in coding and splice region of all these genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*, *MUTYH*, *PTEN*) may relate to the pathogenesis of LS according to their mutant type. Among these mutations, a novel heterozygous insertion (c.420dup, p.Met141Tyrfs) in *MSH2* gene [NCBI Reference sequence NM_000251.2] was found in the mutation list. Compared with other SNPs

in these genes, this mutation has not been reported in the literature to be associated with colorectal pathogenicity in pedigree, and the frequency of this mutation has not been reported, too. The whole process of screening candidate SNPs for LS is shown in Figure 4C, and the identified genes were listed in Table S1.

Next, we found that this mutation can result in a frameshift and introduce a stop codon (stop in exon 3) in 10 amino acids downstream. So that this mutation was predicted to produce a truncated *MSH2* protein as Figure 5A presented, and the structure of full-length *MSH2* which predicted by *Phyre2* is showed in Figure 5B. Besides, we found that this mutation is highly conserved across several species (Figure 5C). These results suggest that this mutation may play an important role in the function of the *MSH2* protein.

The Frameshift Mutation Was Found in All Cancer Diagnosed Members of This Pedigree

Sanger sequencing, as the golden standard of variation identifying, was performed on this patient (III-16) to validate some of these variants. As Figure 6A and B showed, our result can be validated by Sanger sequencing. To make sure if this mutation is heritable or not, Sanger sequencing was performed on all other members with tumors (II-3, III-5, III-18, III-20, III-22, III-24 and IV3) in the pedigree. Surprisingly, all these members carry this insertion in *MSH2* (Figure 2 and

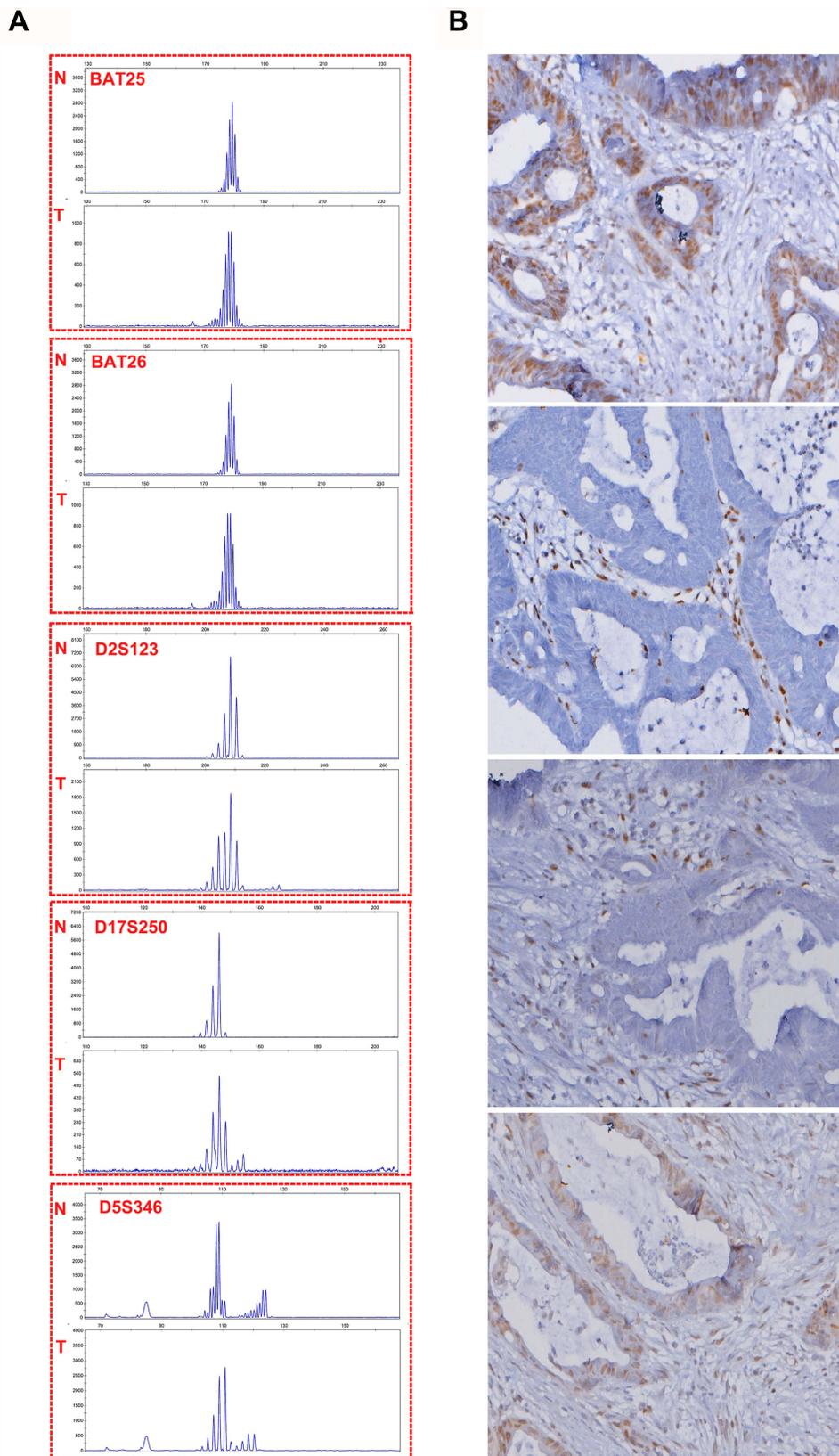


Figure 3 MSI state of the proband's mother (II-3). **(A)** Colon tumor from II-3 showing instability for BAT26, D17S250 and D5S346. **(B)** Immunohistochemical staining of mismatch repair proteins in II-3 are the same as those in III-16: positive nucleus staining for MLH1 and PMS2, while absent of nuclear staining for MSH2 and MSH6 protein in tumor tissue.

Table 2 MMR Related Status of Proband and His Mother

Patient	Probands' Mother Proband II-2	Proband III-16
MSI status	MSI-H	MSI-H
MSI status in panel		
BAT-25	MSS	NA
BAT-26	MSI	NA
D-123	MSS	NA
D-250	MSI	NA
D-346	MSI	NA
IH of MMR proteins		
MLH1	+	+
MSH2	-	-
MSH6	-	-
PMS2	+	+

[Table S1](#)). In other words, this mutation is heritable. Their young offspring also have a great chance to carry this variation. We also performed Sanger sequencing of other mutations on these members. Sequencing results of other mutations were provided in [Table S1](#). Some family members also carry other mutations including *MLH1*(rs1800734), *EPCAM* (rs1126497) and *MUTYH* (rs3219468). The *MLH1* variant (rs1800734), which has been reported to be associated with the risk of several cancers including CRC, glioblastoma, endometrial cancer, and lung cancer.⁴⁵⁻⁴⁸ But II-7 was diagnosed with nasopharyngeal carcinoma. However, no literature has reported that patients with nasopharyngeal carcinoma have been diagnosed with Lynch syndrome until now. And His healthy descendants (III-36 and III-40) also carry this mutation. Moreover, homozygous mutation of this SNP was also found carried by two healthy members (III-20 and IV-5), indicated that this mutation is also present in the general population. In addition, unlike the absence of *MSH2*, our immunohistochemistry (IHC) experiments revealed that the *MLH1* protein is still expressed in the tumor tissues. This result indicated that the *MSH2* is severely affected by the truncated mutation rs1114167810, while *MLH1* is still expressed in this patient. Indeed, the frequency of this mutation in the general population is also as high as 0.24. These evidences indicate that this mutation does not play a major role in the pathogenesis of this family's LS. Similarly, the mutation frequencies of both *EPCAM* (rs1126497) and *MUTYH* (rs3219468) are also quite high, which indicated that these two mutations are unlikely to cause LS. These evidences indicated that

rs1114167810 in *MSH2* might be the leading cause of this family's Lynch syndrome.

The Mutated *MSH2* Causes a Decreased Protein Level and an Increased Sensitivity to Anti-Cancer Drugs in vitro

In order to clarify the effect of the mutation on expression and function of *MSH2*, His-tagged wild-type or mutant *MSH2* constructs were transiently expressed in HCT116 cells. Western blot analysis was carried out using an anti-*MSH2* antibody after 48 h. In transfected cells, a 100-kDa band was detected, which agreed with the size of the full-length *MSH2* protein in control. The mutant *MSH2* showed an obvious decrease in the level of *MSH2* compared with the wild-type protein, since the expression levels of the control and mutant samples were comparable ([Figure 7A](#)). *MSH2* exhibited a markedly lower expression in HCT116 cells with mutant *MSH2* constructs than that with wild-type *MSH2* constructs ([Figure 7B](#)).

Many studies suggested that MMR genes played important roles in chemotherapy.⁴⁹⁻⁵² Is this mutation important for the prediction of chemotherapy? Considering that the proband's chemotherapy regimen is oxaliplatin and 5-fu, and irinotecan is a commonly used chemotherapy drug for colorectal cancer, we use these three drugs for cell viability experiments. Two transfected cells were incubated with different concentrations of 5-FU, Oxaliplatin and Irinotecan. In mutant *MSH2* transfected HCT116, the IC50 value for 5-fluorouracil is lower than that in HCT116 cells transfected with wild-type *MSH2* constructs, although the p-value indicates an insignificant result (17.62 vs 20.49, P>0.05). The IC50 for Oxaliplatin and Irinotecan in HCT116 transfected with mutant *MSH2* constructs is significantly lower than wild-type *MSH2* constructs. (Oxaliplatin, 10.85 vs 15.19, P<0.05; Irinotecan, 29.88 vs 41.84, P<0.05). ([Figure 7C-E](#)). These results indicated that this mutation could decrease the expression of *MSH2* and increase the sensitivity of tumor cells to the anti-cancer drug.

Discussion

Germline deleterious defects in MMR genes, mainly *MLH1*, *MSH2*, *MSH6* and *PMS2* were found to be related with LS.^{21,22} Froggatt et al first independently reported the mutation of MMR genes is associated with Lynch syndrome.⁵³ Nearly all LS and 10%~20% of sporadic colorectal cancer occur gene mutation of

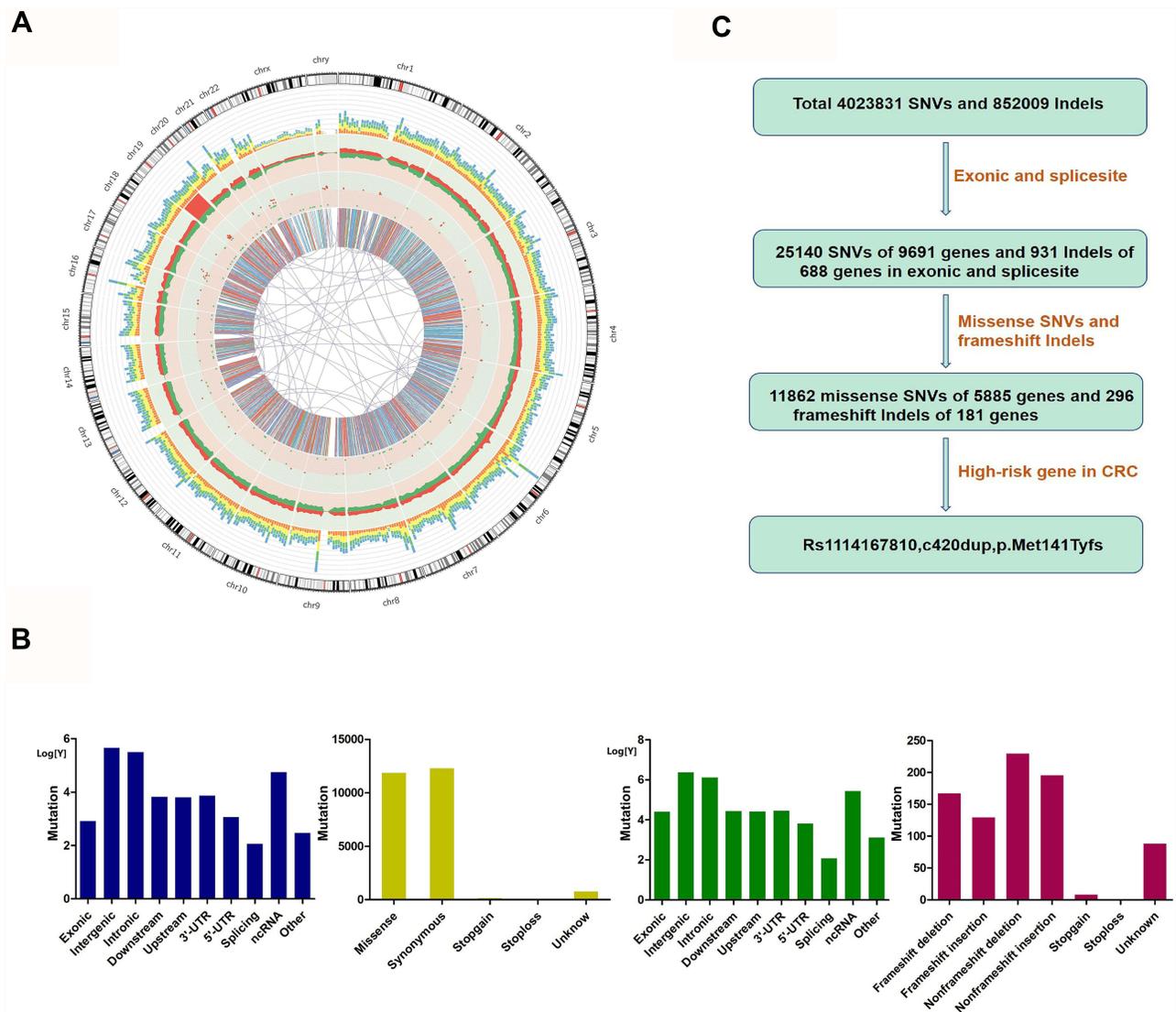


Figure 4 Whole genome sequencing results of the proband (III-16). **(A)** The landscape of all types of variations in this patient. The columns in the first circle indicated the frequency of single nucleotide polymorphism, while red, yellow, green and blue indicated alt allele “A”, “G”, “C” and “T” mutations, respectively. The second circle indicated the frequency of InDels, while red and green indicated insertion and deletion, respectively. The third circle indicated the CNV of this patient, while red and green indicated increased copy number and decreased copy number, respectively. The copy number ranges from 0 to 161. The inner-circle referred to the SV of this patient. **(B)** The distribution of SNVs and InDels in genome regions and the type of mutations in the coding region of the exon. The color of blue and yellow indicated SNVs, and the color of green and red-brown indicated InDels. **(C)** The whole process of screening candidate SNPs for LS.

MMR gene.⁵⁴ MMR genes are associated with several cellular functions including repair the DNA mismatch error, DNA double-strand breaks, DNA destabilization and apoptosis. In other words, the MMR proteins are very significant in maintaining both DNA stability and cell-cycle regulation.^{1,19,20} Immunohistochemical analysis of MMR protein status in the tumor and MSI status can provide useful clues to identify which MMR gene is involved in tumor pathogenesis: indeed, the lack of expression of one of the proteins or the demonstration of MSI-High status might also be relevant in suggesting

a correlation with a pathogenic mutation. In this way, genetic testing of MMR genes can be applied to the diagnosis of LS. So far, a large number of pathogenic MMR gene mutations have been reported to be associated with LS in different countries and ethnic groups, in particular in *MLH1* and *MSH2*.

In the present study, we describe a Chinese pedigree suffering from LS. A novel *MSH2* mutation (rs1114167810, c.420dup, p.Met141Tyrfs) was identified in a 48-year-old LS patient and his family history fulfilled Amsterdam II criteria. We found some candidate gene

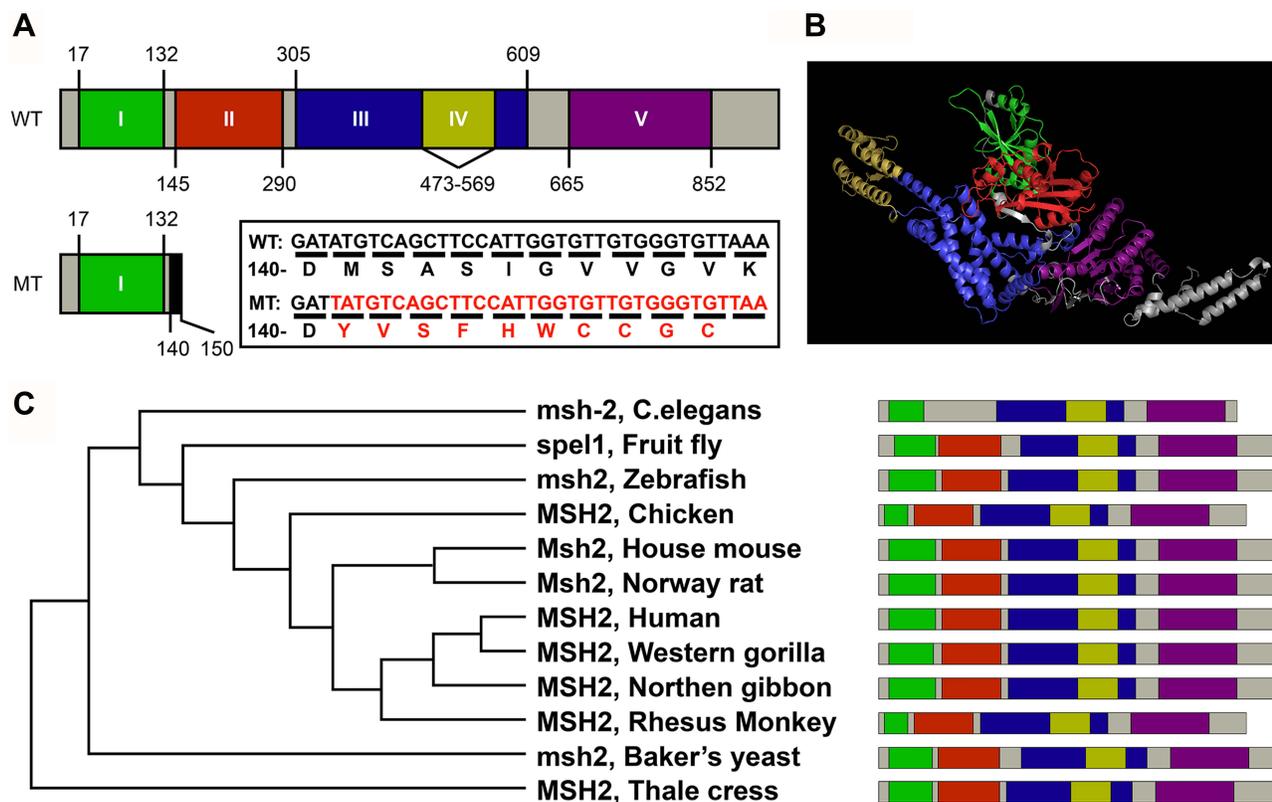


Figure 5 The truncation mutation c.420dup in *MSH2*. Information of this WGS-identified mutation is presented in this figure. **(A)** The sequence and amino acid change caused by this mutation is indicated in red color. This mutation can cause a large part of domains losing and all annotated domains of *MSH2* are marked in different colors. **(B)** The structure of *MSH2* which predicted by *Phyre2* is showed. Different colors indicated different domains as presented in **(A)**. **(C)** The conservation of *MSH2* across species is presented. Different colors in the right panel indicated different domains as presented in **(A)**.

mutations including rs1114167810 through whole genome-wide sequencing at first. Using Sanger sequencing, we verified that this insertion in *MSH2* is carried by this patient. Sanger sequencing results showed that this mutation was found in all LS diagnosed family members, who underwent genetic tests. However, for non-Lynch syndrome member (II-7), we did not detect this mutation in his offspring. This means that the family of II7 did not inherit this pathogenic mutation, and thus did not cause the occurrence of Lynch syndrome. This result was perfectly explained the phenomenon in Figure 2. On the contrary, we found both Lynch syndrome and non-Lynch syndrome families carry *MLH1* mutations (rs1800734). On the other hand, our IHC experiments only revealed the absence of *MSH2* and *MSH6* protein expression rather than *PSM2* and *MLH1* in the tumor tissues. In conclusion, we believe that the decrease of *MSH2* plays a major role in the occurrence of Lynch syndrome in this family, while this truncated mutation rs1114167810 is the main causer. As for other found

mutations with high frequency, we speculated that these variants did not play a major role in the development of LS. We also found that some members in this family carried three candidate variants in tumor-related genes: *PTEN* (rs71022512), *PTEN* (rs5786797) and *PMS2* (rs374762935). But these tandem repeat mutations may be caused by the insertion of a mismatch repair gene. This assumption was not carried in this study and required further verification.

Although we did not experimentally confirm that this variant causes a truncated protein, we predicted that this variant would lead to the premature termination of codon production. The IHC result and our functional experiment also showed that the *MSH2* (c.420dup, p. Met141Tyrfs) variant can decrease the expression of proteins and mRNA. We also investigated the effect of this variant on the response of colorectal cancer cells to clinically used chemotherapy drugs in this study. We found that this variant causes tumor cells (HCT116) to be sensitive to oxaliplatin and irinotecan, but has no

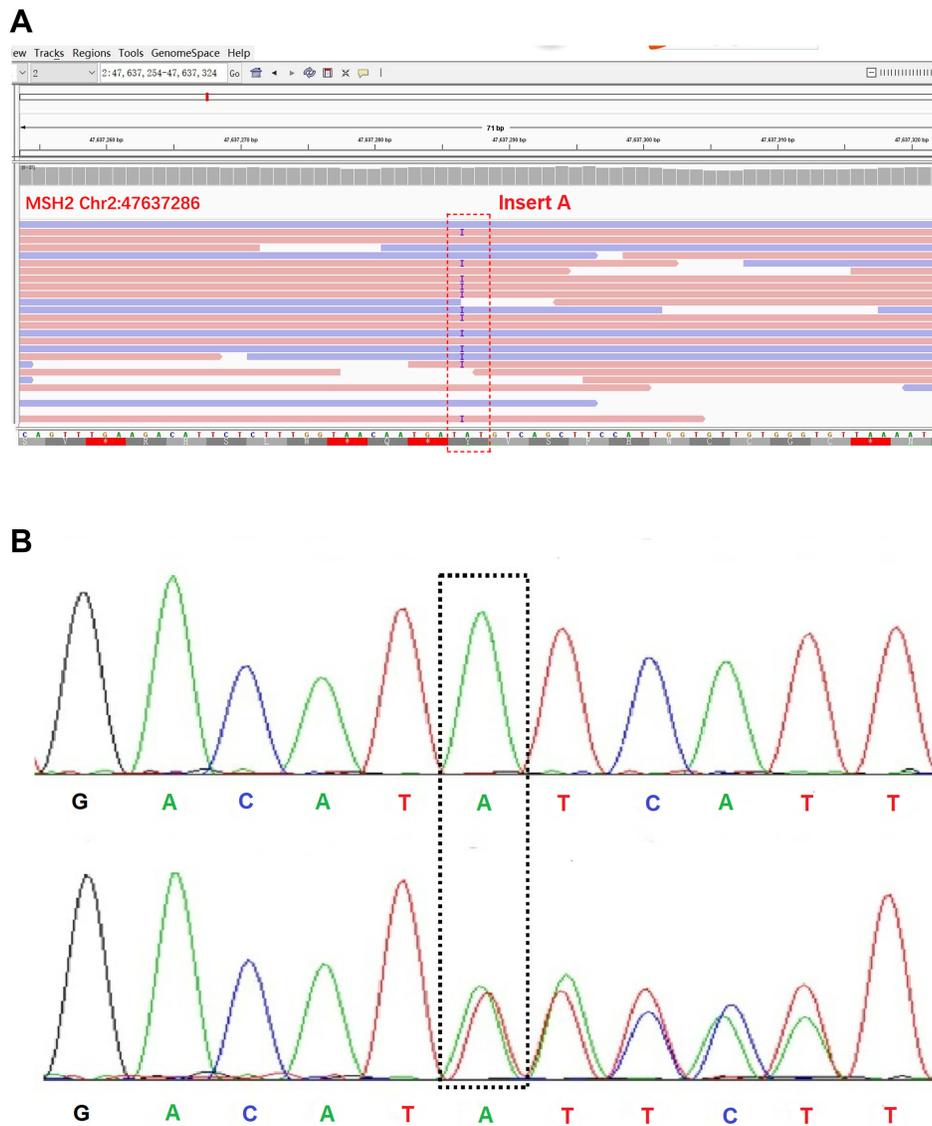


Figure 6 Identification of this truncation mutation c.420dup in *MSH2*. **(A)** Visualization of *MSH2* sequencing reads containing c.420dup mutation with the Integrative Genomics Viewer (IGV), the red box represents the insertion of the A base. **(B)** Confirmation of c.420dup mutation in the proband and His family members by Sanger sequencing.

effect on 5-fu sensitivity, which should be further validated in a clinical study. This is basically consistent with what has been previously described for Lynch syndrome-related 11 colorectal cancer patients by Maccaroni et al, although this mutation is not referred in 12 this study, perhaps because of its extremely low allele frequency. In addition, other literatures also supported that MMR deficient was associated with poor benefit from 5-FU treatment in CRC.^{51,55–57} In contrast, MMR deficiency sensitives CRC cells to oxaliplatin.^{58–61} Oxaliplatin's specific 1,2-diaminocyclohexane (DACH) ligand prevents the MMR complex from binding to its DNA adduct, resulting in loss of

repair capacity and subsequent apoptosis of tumor cells.⁵⁸ But the clinical results about the relationship between MMR status and CPT-11 were inconclusive, Charara et al demonstrated that high microsatellite instability with loss of mismatch repair protein is predictive of an improved response to neoadjuvant treatment with 5-FU, CPT-11 and radiation therapy,⁶² while some evidences indicating that MMR status was not associated with the response to CPT-11 in CRC treatment.³⁶ All in all, this means that this mutation may also serve as a target for a patient to predict chemotherapy response, which requires subsequent studies in the clinic to further confirm.

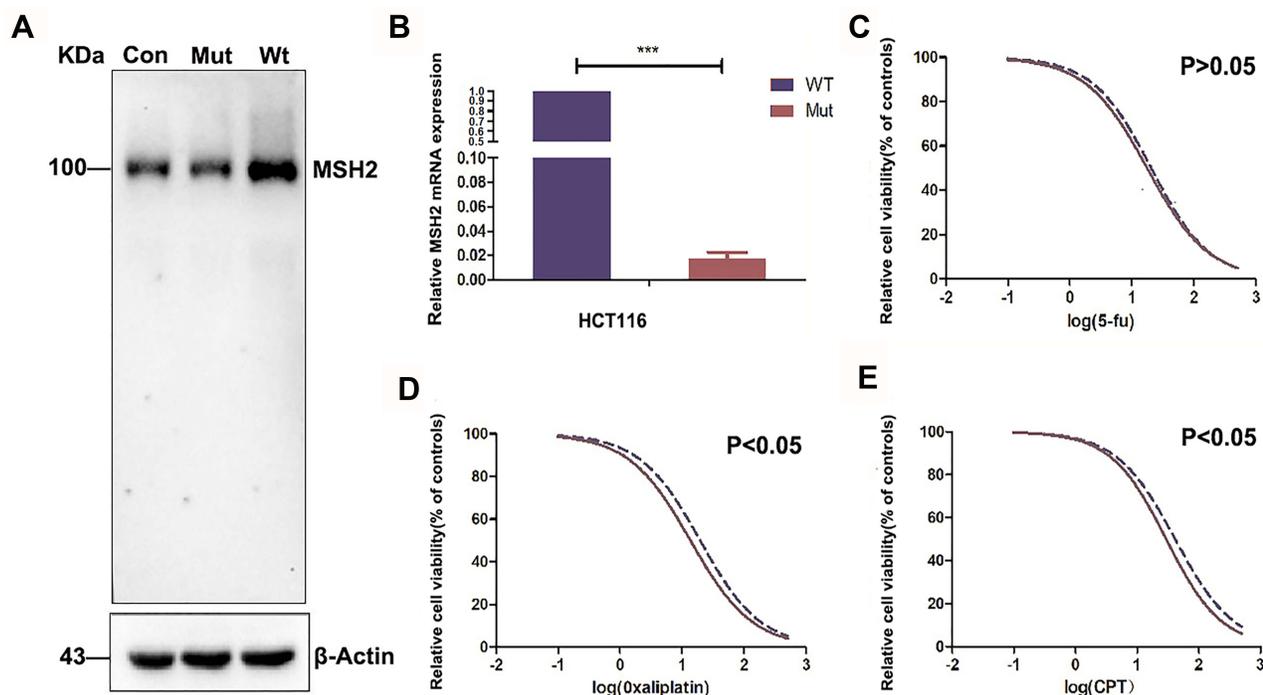


Figure 7 Effect of this truncation mutation c.420dup in *MSH2* on the expression of *MSH2* expression and cellular response to chemotherapy drugs. **(A)** The *MSH2* expression level in transfected colorectal cancer cells (HCT116). **(B)** Expression level of *MSH2* mRNA in HCT116 cells transfected with wild-type and c.420dup mutant *MSH2* constructs. The data are shown the mean of $2^{-\Delta\Delta Ct}$. **(C)** Dose–response analyses of 5-fluorouracil effect on inhibition of viable cell numbers (cell viability) were carried out in HCT116 cells which were transfected with wild-type and c.420dup mutant *MSH2* constructs. The curve consisted of three independent experiments. **(D)** Dose–response analyses of Oxaliplatin effect on inhibition of viable cell numbers (cell viability) were carried out in HCT116 cells which were transfected with wild-type and c.420dup mutant *MSH2* constructs. The curve consisted of three independent experiments. **(E)** Dose–response analyses of Irinotecan effect on inhibition of viable cell numbers (cell viability) were carried out in HCT116 cells which were transfected with wild-type and c.420dup mutant *MSH2* constructs. The curve consisted of three independent experiments.

Altogether, our experimental evidence supports a pathogenic role for the *MSH2* c.420dup mutation, reinforcing the importance of the variants in offering genetic counseling management, and surveillance in LS families.

Ethical Statement

This study is conducted in accordance with the declaration of Helsinki and is approved by the ethics committee of Xiangya Hospital, the trial number is 2018121120.

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Disclosure

The authors declare no conflict of interest in this work.

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