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Editorial Board Member of *World Journal of Clinical Cases*, Manel Sabate, MD, PhD, Associate Professor, Interventional Cardiology Department, Clinic University Hospital, Barcelona 08036, Spain

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EDITORS-IN-CHIEF
Sandro Vento, MD, Department of Internal Medicine,
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Must Peutz-Jeghers syndrome patients have the *LKB1/STK11* gene mutation? A case report and review of the literature

Fu-Xiao Duan, Guo-Li Gu, Hai-Rui Yang, Peng-Fei Yu, Zhi Zhang

Fu-Xiao Duan, Guo-Li Gu, Hai-Rui Yang, Peng-Fei Yu, Zhi Zhang, Department of General Surgery, Air Force General Hospital of Chinese PLA, Beijing 100142, China

ORCID numbers: Fu-Xiao Duan (0000-0002-3224-9017); Guo-Li Gu (0000-0002-9998-047X); Hai-Rui Yang (0000-0003-2768-8493); Peng-Fei Yu (0000-0002-0528-1839); Zhi Zhang (0000-0001-5870-1940).

Author contributions: Gu GL designed the research; Duan FX, Gu GL, Yang HR and Yu PF prepared the samples for sequencing; Duan FX and Zhang Z conducted the sequencing; Gu GL and Duan FX collected and analyzed the data; Duan FX wrote the manuscript; Gu GL revised the manuscript.

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Correspondence to: Guo-Li Gu, MD, MSc, Associate Professor, Department of General Surgery, Air Force General Hospital of Chinese PLA, No. 30, Fucheng Road, Haidian District, Beijing 100142, China. kzggl@163.com

Telephone: +86-10-66928303

Fax: +86-10-66928303

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Abstract

Peutz-Jeghers syndrome (PJS) is an autosomal dominant inherited disease, which is characterized by mucocutaneous pigmentation and multiple gastrointestinal hamartoma polyps. The germline mutation of *LKB1/STK11* gene on chromosome 19p13.3 is considered to be the hereditary cause of PJS. However, must a patient with PJS have the *LKB1/STK11* gene mutation? We here report a case of a male patient who had typical manifestations of PJS and a definite family history, but did not have *LKB1/STK11* gene mutation. By means of high-throughput sequencing technology, only mutations in *APC* gene (c.6662T > C: p.Met2221Thr) and *MSH6* gene (c.3488A > T: p.Glu1163Val) were detected. The missense mutations in *APC* and *MSH6* gene may lead to abnormalities in structure and function of their expression products, and may result in the occurrence of PJS. This study suggests that some other genetic disorders may cause PJS besides *LKB1/STK11* gene mutation.

Key words: Peutz-Jeghers syndrome; Gastrointestinal polyps; High-throughput sequencing; *LKB1/STK11*; *APC*; *MSH6*; Hamartoma

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Core tip: The germline mutation of *LKB1/STK11* gene on chromosome 19p13.3 is considered to be the hereditary cause of Peutz-Jeghers syndrome (PJS). We report a male PJS patient, who had typical manifestations and a definite family history, and but did not have *LKB1/STK11* gene mutation. By means of high-throughput sequencing technology, only mutations in *APC* gene (c.6662T > C: p.Met2221Thr) and *MSH6* gene (c.3488A > T: p.Glu1163Val) were detected in this case. This study suggests that some other genetic disorders may cause PJS besides *LKB1/STK11* gene mutation.

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INTRODUCTION

Peutz-Jeghers syndrome (PJS) is an autosomal dominant inherited disease characterized by mucocutaneous pigmentation and multiple gastrointestinal hamartoma polyps^[1]. PJS is a very rare disease, with an incidence of about 1/25000^[2]. It has prominent clinical manifestations, and serious clinical hazards since the gastrointestinal hamartoma polyps of PJS can lead to severe complications, such as gastrointestinal bleeding, bowel obstruction, intussusception and cancerization. PJS hamartoma polyps are mainly distributed in the small intestine (especially the proximal jejunum), so PJS has a persistent disease course and concealing lesion site^[3-5]. This may cause mental stress and economic burden on the patients and their families. Therefore, it is necessary for us to study PJS.

The germline mutation of *LKB1/STK11* gene on chromosome 19p13.3 is considered as the hereditary cause of PJS^[6,7]. As a tumor suppressor gene, *LKB1/STK11* contains 9 exons and 11 introns. At present, the detected mutation rate of *LKB1/STK11* gene is 80%-94% by direct sequencing technology and multiplex ligation-dependent probe amplification (MLPA)^[8-10]. This may suggest that the genetic etiology of PJS is heterogeneous^[11-13]. Are there any other genetic causes for PJS besides *LKB1/STK11* gene? Here, we report a case of male patient with PJS who had missense mutations of *APC* gene and *MSH6* gene, but without mutations of *LKB1/STK11* gene.

CASE REPORT

Patient information

A 32-year-old male patient with PJS was hospitalized for endoscopic polypectomy in January 19, 2015. He had black spots on lips, buccal mucosa and fingertips since the age of 7 (Figure 1), and intermittent abdominal pain and black stool since the age of 25. He was

diagnosed with PJS and underwent multiple endoscopic polypectomies to remove hamartoma polyps in the gastrointestinal tract *via* gastroscopy, enteroscopy and colonoscopy. His father was also diagnosed with PJS. This patient was in full compliance with the clinical diagnostic criteria for PJS issued by the WHO^[14]: Patients without a family history can be diagnosed with PJS if they have three or more histopathologically confirmed PJS polyps, or characteristic, notable, mucocutaneous pigmentation with any amounts of PJS polyps. Patients with a family history can be diagnosed with PJS if they have any amounts of PJS polyps, or characteristic, notable, mucocutaneous pigmentation.

The abdominal and pelvic CT scan showed that there were many different sizes of polyps distributed in the descending duodenum and group 3rd-group 6th of the small intestine, and the local intestinal canal was torsional (Figure 2). Transanal double-balloon electronic enteroscopy revealed that there were multiple long pedicle, subpedicle or sessile polyps sized 0.5-1.2 cm in the colorectum, especially the descending colon, sigmoid colon and rectum. In addition, there were many huge lobulated polyps at a distance of 40 cm from the ileocecal valve that caused intestinal tract blockage. The patient underwent laparoscopic exploration, intussusception reduction, endoscopic small intestinal polyp cauterization, and small intestinal multiple hamartoma resection in January 25, 2015 (Figure 3). Postoperative pathological report showed PJS hamartoma polyps without canceration (Figures 4 and 5).

DNA extraction, quantification and quality control

We used paraffin tissue DNA extraction kit to extract DNA from the polyp tissue according to the instructions of the kit (QIAamp Tissue DNA FFPE Tissue Kit, QIAGEN, QIAGEN Strasse 1407124 Hilden, QIAGEN). Preliminary screening of the sample DNA was carried out using the peak map, and purity test was done through the ultraviolet spectrophotometer (Nanodrop 2000, Thermo Scientific, Wilmington, DE, United States). Accurate quantitative analysis of complete DNA fragments in the sample DNA was performed using the fluorescence quantitative instrument (The Qubit 2 Fluorometer, Thermo Scientific, Wilmington, DE, United States). The results showed that the concentration of the sample DNA (A260/A280 2.01) was 1314 ng/μL, and the concentration of the complete DNA fragments was 160 ng/μL, which was higher than the requirement of 10 ng/μL.

cDNA libraries construction and sequencing

We constructed cDNA libraries with Ion AmpliSeq Library Kit 2 according to the instructions of the manufacturer (Life 5791, Van Allen, Way Carlsbad. CA, United States). Ion AmpliSeq Custom (IAD72340_182_pool 1, IAD72340_182_pool 2) (Life Technologies) was used to prepare a gene panel consisting of a primer pool for the entire coding region and exon-intron boundaries in 14 genes which often mutated in hereditary colorectal



Figure 1 Mucocutaneous pigmentation of the patient.

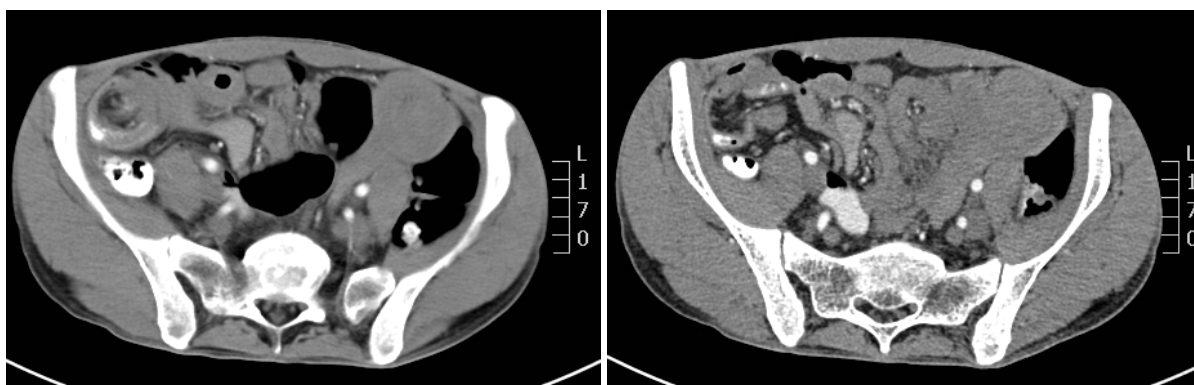


Figure 2 Abdominal and pelvic CT scan. It showed that many polyps are distributed in the descending duodenum and the small intestine, and the local intestinal canal was torsional.

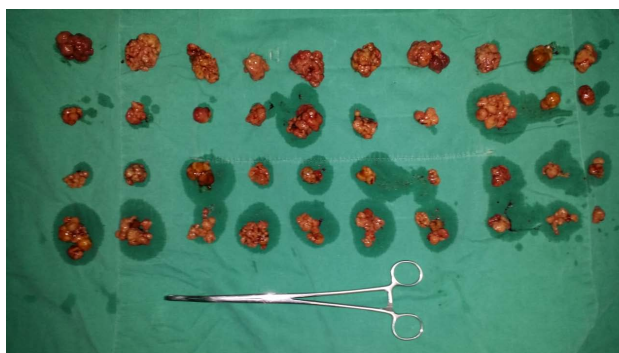


Figure 3 Hamartoma polyps removed from the patient's gastrointestinal tract.

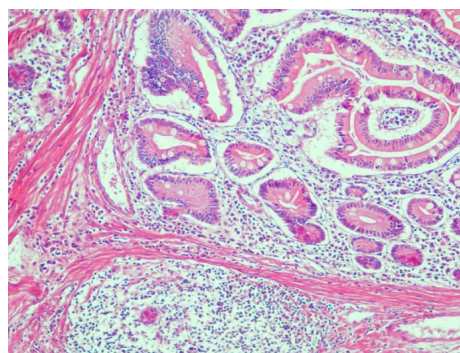


Figure 4 Postoperative pathological reports showed Peutz-Jeghers syndrome hamartoma polyps (HE, original magnification $\times 200$).

cancer (*APC*, *AXIN2*, *BMPR1A*, *EPCAM*, *MLH1*, *MLH3*, *MSH2*, *MSH6*, *MUTYH*, *PMS1*, *PMS2*, *PTEN*, *SMAD4*, *LKB1/STK11*). After amplification, the libraries were purified using paramagnetic particle method (AMPure XP reagent, Beckman, United States). The fluorescence quantitative PCR instrument (ViiA 7 Dx, Life, Technologies Holdings Pte Ltd Block, Singapore) was used to detect the quantitative index of the libraries. Then the template was dealt with Ion OneTouch 2 template system (Life Technologies, Carlsbad, CA, United States) for preparation (Ion OneTouch2) and enrichment (Ion OneTouch ES). Finally, the high-

throughput sequencing was performed on Ion PGM (Life, Technologies Holdings Pte Ltd Block, Singapore) using Ion 316 CHIP (Life Technologies Corp, Carlsbad, CA, United States).

Statistical analysis

The quality control parameters of the sequencing data were as follows: percentage reads on target $> 85\%$, uniformity of base coverage $> 85\%$, average base coverage depth: $200 \times -500 \times$. The data were analyzed by Torrent Suite software (Life Technologies, v5.0.4) and compared with database of hg19 human

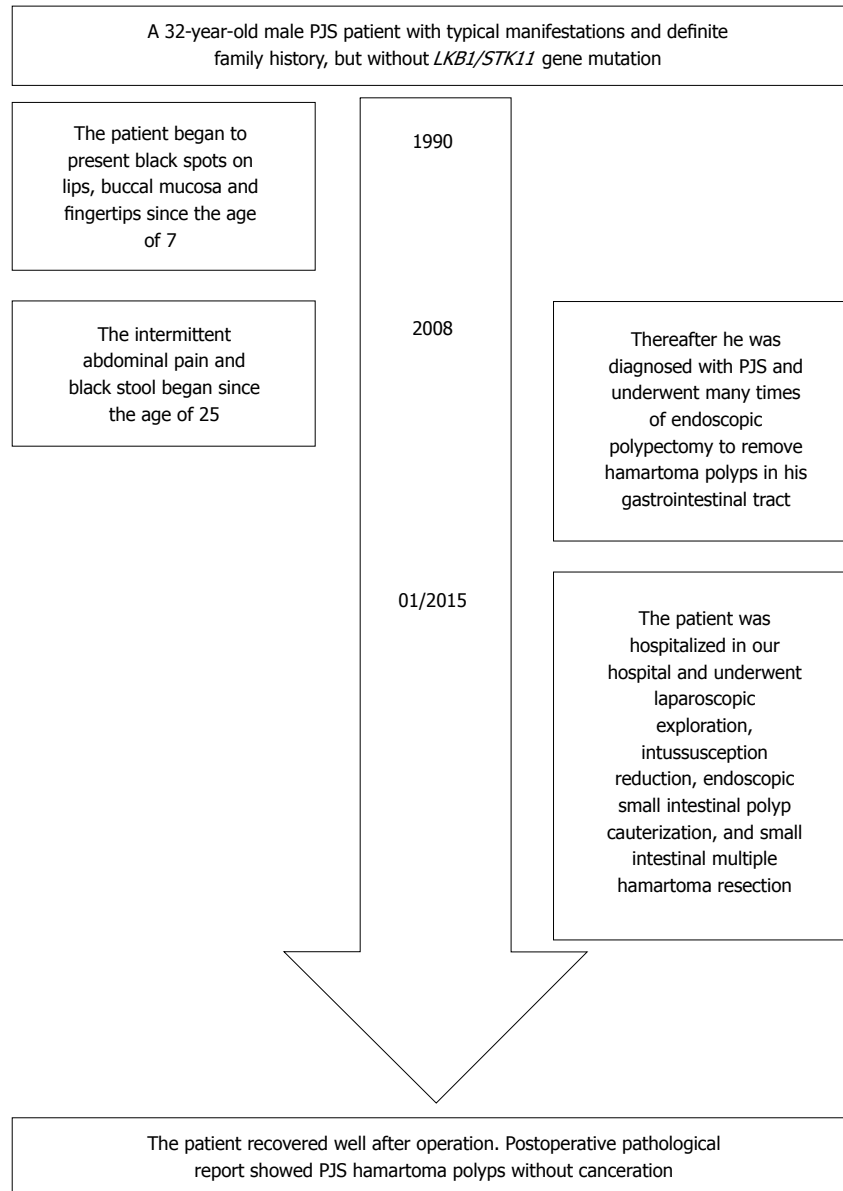


Figure 5 Timeline of the patient. PJS: Peutz-Jeghers syndrome.

reference genome. The detected gene mutations were annotated by Ion Reporter software (<https://ionreporter.lifetechnologies.com/ir/secure/home.html>) and ANNOVAR package (<http://wannovar.wglab.org/>).

Preliminary screening of mutation sites for verification was carried out according to the mutation frequency. Allele frequency database of the herd was based on the dbSNP database (<http://www.ncbi.nlm.nih.gov/projects/SNP/>), 1000 Genomes Project (<http://ftp.ncbi.nlm.nih.gov/>) and the genome aggregation database (gnomAD, <http://gnomad.broadinstitute.org/>). According to the HGMD (version 2017.03, <http://www.hgmd.cf.ac.uk/ac/index.php>), mutation sites of minor allele frequency < 0.01 and the suspected or pathogenetic sites with frequency of 0.01-0.05 were retained for verification.

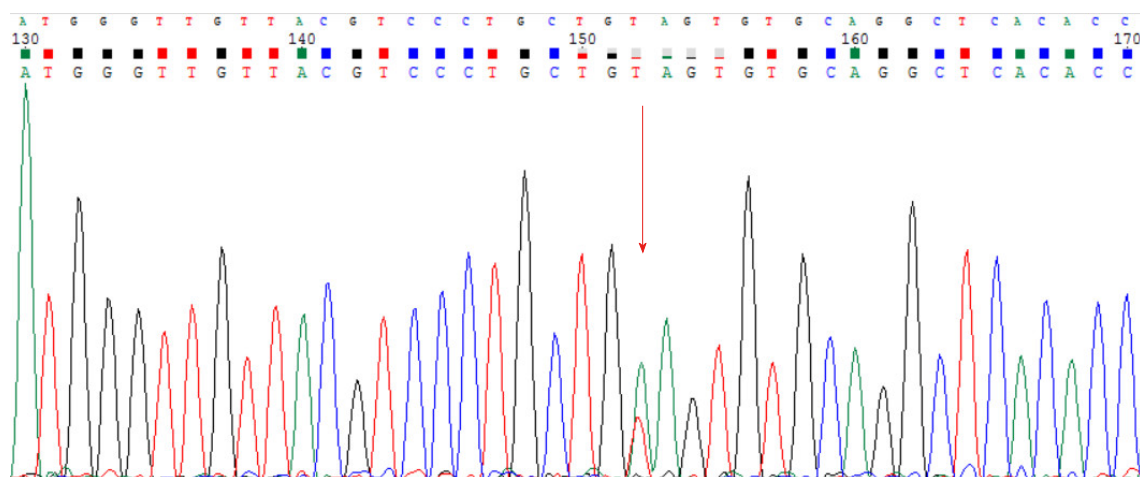
The Sanger sequencing technology was applied to candidate sites verification. Protein function was

predicted with the software Polymorphism Phenotyping V2 (PolyPhen-2, <http://genetics.bwh.harvard.edu/pph2/index.shtml>), MutationTaster (<http://www.mutationtaster.org/>), functional analysis was performed using the Hidden Markov Models (FATHMM, <http://fathmm.biocompute.org.uk/index.html>) and Mendelian Clinically Applicable Pathogenicity (M-CAP, <http://bejerano.stanford.edu/MCAP/>). GERP++ (<http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html>) and PhyloP (<http://compugen.bscb.cornell.edu/phast>) were used to forecast the conservatism of the impaired amino-acid residues.

Quality control data

This sequencing could read 28269827 bases. The base number of Q20 was 24754457, accounting for 87.56%. Percentage reads on target was 92.12%. Average base coverage depth was $286 \times$ (coverage $\geq 20 \times$

A



B

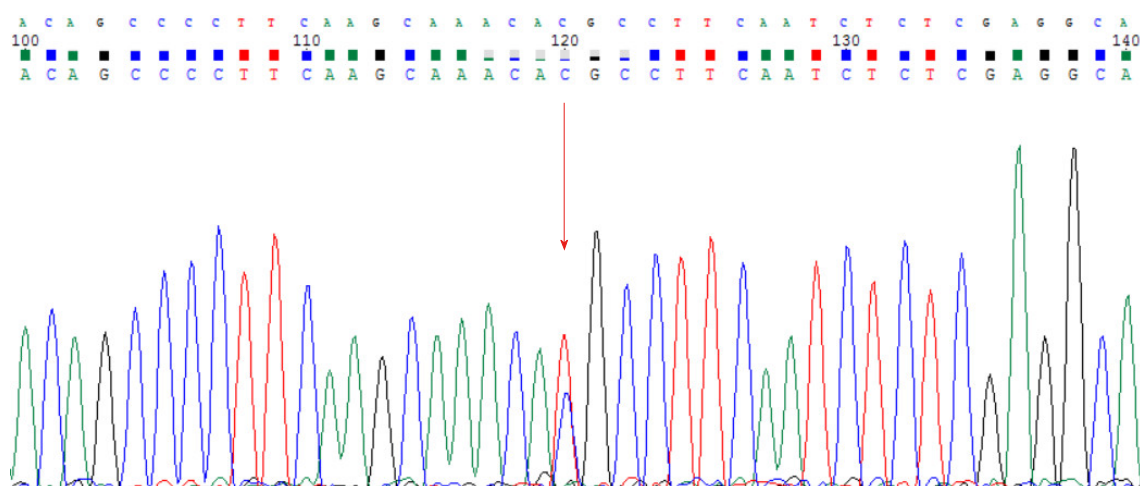


Figure 6 Results of the Sanger sequencing. Mutation sites are marked with arrows. A: *MSH6*: c.3488A>T; B: *APC*: c.6662T>C.

94.39%, coverage $\geq 100 \times 77.38\%$, coverage $\geq 500 \times 12.75\%$). Uniformity of base coverage was 92.12%.

Sequencing results

No mutation of *LKB1/STK11* gene was found in this patient, but he carries missense mutations in exon 6 of *MSH6* gene and exon 16 of *APC* gene (*MSH6*: NM_000179.2: exon6: c.3488A>T: p.Glu1163Val; *APC*: NM_000038.5: exon16: c.6662T>C: p.Met2221Thr) (Figure 6). The Sanger sequencing results proved the existence of these mutations (primers: *APC*: forward primer: CCAGGGGAGAAAAGTACATTGGA, reverse primer: ACTTGACTTGAGGAGCTATTTTCG; *MSH6*: forward primer: ACAGAACCAACGTACATGTGA, reverse primer: TTCTGTCTGAGGCACCAAGTC).

Prediction of protein function and amino-acid residue conservatism

The changes in the structure and function of *APC* protein caused by gene mutations were analyzed and determined as benign by Polyphen-2, as disease caused by MutationTaster, and as damage by FATHMM and by

M-CAP. Evolutionary conservative analysis software of GERP++ and phyloP showed that the impaired amino-acid residue was conserved in different species, suggesting that the mutation may be pathogenic.

The structure and function changes of the mutated *MSH6* protein were analyzed and defined as possible damage or benign by Polyphen-2, as disease caused by MutationTaster and as damage by FATHMM. Evolutionary conservative analysis software of GERP++ and phyloP indicated that the mutant amino-acid residue was most evolutionarily conserved and was likely pathogenic (Tables 1 and 2).

DISCUSSION

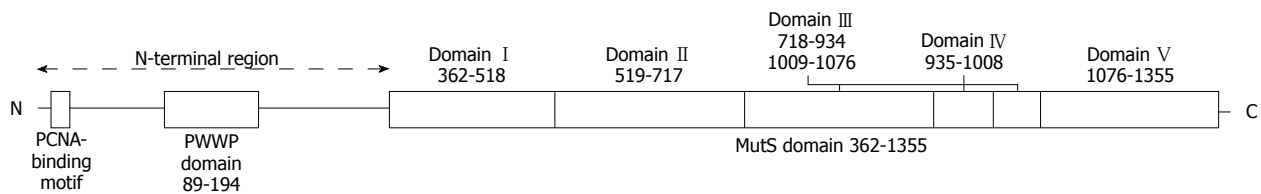
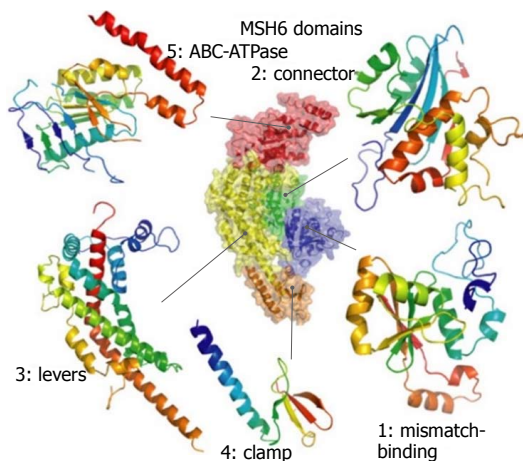
In this study, the patient had a clear family history and typical clinical manifestations of PJS, and histologic examination of his polyps proved to be hamartoma. However, we only detected the missense mutations in the *APC* gene and *MSH6* gene from his polyp tissue by high-throughput sequencing and Sanger sequencing technology, but no mutation in the *LKB1/STK11* gene.

Table 1 Prediction of protein function using different softwares

| Gene | Polyphen-2_HDIV | | Polyphen-2_HVAR | | MutationTaster | | FATHMM | | M-CAP | |
|------|-----------------|-------------------|-----------------|------------|----------------|-----------------|--------|------------|-------|------------|
| | Score | Prediction | Score | Prediction | Score | Prediction | Score | Prediction | Score | Prediction |
| MSH6 | 0.670 | Possibly damaging | 0.411 | Benign | 1 | Disease causing | -2.12 | Damaging | - | - |
| APC | 0.156 | Benign | 0.026 | Benign | 0.737 | Disease causing | -2.47 | Damaging | 0.046 | Damaging |

Table 2 Prediction of amino-acid residue conservatism

| Gene | Exon | Protein | Coding | GERP++ | | PhyloP | |
|------|------|--------------|-------------|--------|------------|--------|------------|
| | | | | Score | Prediction | Score | Prediction |
| MSH6 | 6 | p.Glu1163Val | c.3488A > T | 5.23 | Conserved | 8.923 | Conserved |
| APC | 16 | p.Met2221Thr | c.6662T > C | 6.02 | Conserved | 3.925 | Conserved |

**Figure 7** Domain organization of human *MSH6*^[26]. It mainly consists of PCNA-binding motif, PWWP domain and the MutS domain.**Figure 8** The domain structure of *MSH6*. Figures were generated with PyMOL^[29].**Figure 9** Structure of the mutant *MSH6* (p.Glu1163Val). Arrow indicates the position of the mutation. The figure was generated with Swiss-Model online software.

The mutation detected in *APC* gene has not been reported previously, and the MAF was 8.3×10^{-6} according to the ExAC database. The mutation detected in *MSH6* gene has been reported in some sequencings^[15-17], the MAF was 0.0012 according to the ExAC database as well as 0.0028 from 1000G database.

The DNA mismatch repair proteins (MMR), including MLH1, MSH2, MSH3, MSH6, PMS1 and PMS2, could remain gene stability by means of discovering and repairing mismatched bases and insertion/deletion loops improperly incorporated during DNA synthesis^[18-21]. Expression deletion of MMR protein can be found in about 15%-25% sporadic tumors of various tissues^[22]. The *MSH6* gene is located on chromosome 2p16.3, which contains 24 000 base pairs and 10 exons. The *MSH6* protein was first reported as G/T mismatch

repair binding protein (GTBP) in 1995, it constitutes MutS α complex with MSH2^[23-25]. The main domains of *MSH6* protein from N-terminal to C-terminal included (Figure 7): PCNA-binding motif, PWWP domain and the MutS domain^[26-28]. The MutS domain is composed of five parts: (1) Mismatch binding domain, including the 362-518th amino acids; (2) connector domain, including the 519-717th amino acids; (3) levers domain, including the 718-934th and 1009-1075th amino acids; (4) clamp domain, including the 935-1008th amino acids; (5) ABC-ATPase domain, including the 1076-1355th amino acids^[29] (Figure 8). The gene mutation (c.3488A>T) detected in our patient led the 1163rd amino acid of glutamate to become valine in the *MSH6* protein (Figure 9). Changing an acidic amino-acid into a non-polar hydrophobic amino-acid may cause the ABC-ATPase

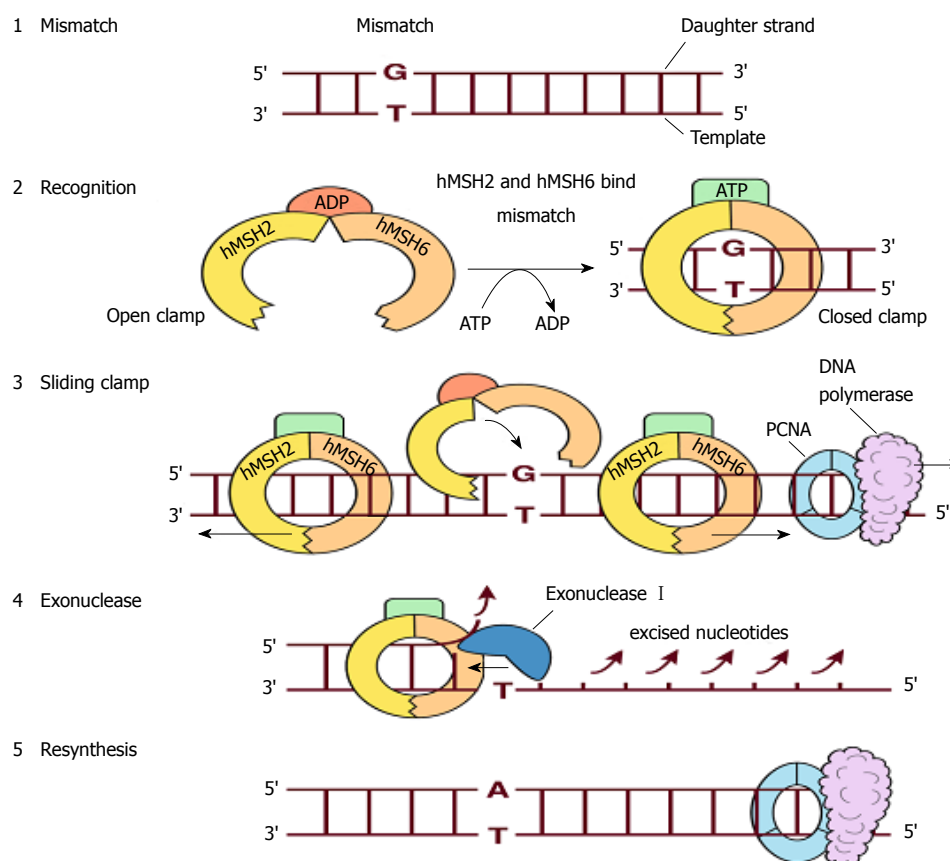


Figure 10 Repairing single-base mismatch in S phase by MutS α ^[30]. 1: A mismatch appears in the daughter strand; 2: Upon encountering the mispair, the MutS α , which consists of hMSH2, hMSH6 and ADP, switches to a closed, sliding clamp along the DNA. This process is accompanied by exchanging of ATP for ADP; 3: Multiple MutS α clamps may be recruited to the mismatch. Moving in the 5' > 3' direction, the MutS α will meet and displace the PCNA-DNA polymerase complex in DNA synthesis; 4: Exonuclease I excises the nucleotides of the daughter strand back to the site of the mismatch; 5: The daughter strand is resynthesized and the error is corrected.

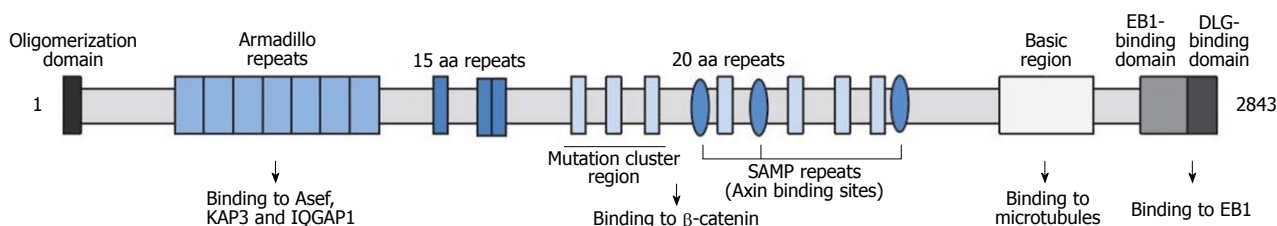


Figure 11 Major structure and functions of APC^[35]. It is made up of oligomerization domain, armadillo repeats domain, 15-amino acid or 20-amino acid repeats domain, SAMP repeats domain, mutation cluster region, basic region, EB1-binding domain and DLG-binding domain.

domain conformational change and affect the hydrolysis of ATP or ADP to ATP conversion process. This change could disorder the open and close conformational transition of the MutS α complex and eventually lead to the loss of MMR expression (Figure 10).

The *APC* gene is located on the autosome 5q21. Its main functions include inhibiting cell proliferation, promoting differentiation, promoting apoptosis, participating in cell migration, cell adhesion and chromosome separation. It is generally believed that the mutation of *APC* gene is an important initiator for the occurrence and development of colorectal cancer^[30,31]. Mutated *APC*

can also be seen in some brain and breast cancers^[32,33]. The molecular weight of APC protein is about 312 kDa, and its N-terminal domains mainly include: (1) Oligomerization domain; (2) armadillo repeats domain; (3) 15-amino acid or 20-amino acid repeats domain; (4) SAMP repeats domain and Mutation cluster region (MCR); (5) Basic region for binding microtubule or f-actin, including the 2219-2580th amino acids; (6) EB1-binding domain; (7) DLG-binding domain^[34,35] (Figure 11). APC protein can participate in the composition of a complex promoting the destruction of β -catenin (a transcriptional permissive factor), and then negatively

regulate the classical Wnt signal pathway. Its C-terminal region, especially the 2200-2400th amino-acid, is rich in bases that could boost the binding process of microtubules^[36-38]. APC protein of cell periphery plays an important role in binding microtubule network, establishing cell polarity and inhibiting invasion according to many studies^[39,40]. A research based on a zebrafish model suggests that cell differentiation may be related to the Warburg effect mediated by APC^[41]. In addition, the differentiation mechanism of human intestinal epithelial cells may be similar to that of zebrafish^[42]. In this case, the mutation (c.6662T>C) causes 2221st methionine of the APC protein to become threonine, which may result in the protein conformation change, and affect cell differentiation, adhesion and migration.

In summary, we detected *MSH6* and *APC* gene mutations in a case of PJS by high-throughput sequencing technology, but failed to detect mutations in *LKB1/STK11* gene, and the mutation of *APC* gene (c.6662T>C: p.Met2221Thr) had never been reported previously. This suggests that mutations of *MSH6* and *APC* genes may affect the occurrence and development of PJS by affecting the MMR pathway, cell differentiation, adhesion and migration. Our research may be helpful in expanding the mutation spectrum of PJS and revealing its genetic heterogeneity.

ARTICLE HIGHLIGHTS

Case characteristics

A 32-year-old male patient with Peutz-Jeghers syndrome (PJS).

Clinical diagnosis

Peutz-Jeghers syndrome.

Endoscopic diagnosis

Multiple long pedicle, subpedicle or sessile polyps sized 0.5-1.2 cm appeared in the colorectum, especially the descending colon, sigmoid colon and rectum.

Imaging diagnosis

Many polyps of different sizes were distributed in the descending duodenum and the 3rd to 6th segments of the small intestine, and the local intestinal canal was torsional.

Treatment

The patient underwent a combined laparoscopic and endoscopic surgery.

Pathological diagnosis

PJS hamartoma polyps without canceration.

High-throughput sequencing

Instead of *LKB1/STK11* gene, we detected missense mutations of *APC* gene and *MSH6* gene.

Experiences and lessons

This research may be helpful in expanding the mutation spectrum of PJS and revealing its genetic heterogeneity.

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