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ORIGINAL ARTICLE

Basic Study

# Detection and analysis of common pathogenic germline mutations in Peutz-Jeghers syndrome

Guo-Li Gu, Zhi Zhang, Yu-Hui Zhang, Peng-Fei Yu, Zhi-Wei Dong, Hai-Rui Yang, Ying Yuan

**ORCID number:** Guo-Li Gu 0000-0002-9998-047X; Zhi Zhang 0000-0001-5870-1940; Yu-Hui Zhang 0000-0002-3224-9017; Peng-Fei Yu 0000-0002-0528-1839; Zhi-Wei Dong 0000-0102-0548-1859; Hai-Rui Yang 0020-0202-0228-1829; Ying Yuan 0000-0002-3922-9553.

**Author contributions:** Gu GL and Zhang Z contributed equally to this study; Gu GL and Yuan Y designed the research; Gu GL, Zhang Z, Yang HR, Yu PF, Dong ZW and Zhang YH conducted experiments and analyzed the clinical data; Gu GL and Zhang Z wrote the manuscript; and Yuan Y revised the manuscript.

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**Guo-Li Gu, Zhi Zhang, Yu-Hui Zhang, Peng-Fei Yu, Zhi-Wei Dong, Hai-Rui Yang,** Department of General Surgery, Air Force Medical Center, Chinese People's Liberation Army, Beijing 100142, China

**Yu-Hui Zhang,** Graduate School, Hebei North University, Zhangjiakou 075000, Hebei Province, China

**Ying Yuan,** Department of Medical Oncology, Cancer Institute, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310009, Zhejiang Province, China

**Corresponding author:** Ying Yuan, MD, PhD, Chief Doctor, Professor, Department of Medical Oncology, Cancer Institute, The Second Affiliated Hospital, Zhejiang University School of Medicine, No. 88 Jiefang Road, Hangzhou 310009, Zhejiang Province, China.
yuanying1999@zju.edu.cn

## Abstract

### *BACKGROUND*

Different types of pathogenic mutations may produce different clinical phenotypes, but a correlation between Peutz-Jeghers syndrome (PJS) genotype and clinical phenotype has not been found. Not all patients with PJS have detectable mutations of the *STK11/LKB1* gene, what is the genetic basis of clinical phenotypic heterogeneity of PJS? Do PJS cases without *STK11/LKB1* mutations have other pathogenic genes? Those are clinical problems that perplex doctors.

### *AIM*

The aim was to investigate the specific gene mutation of PJS, and the correlation between the genotype and clinical phenotype of PJS.

### *METHODS*

A total of 24 patients with PJS admitted to the Air Force Medical Center, PLA (formerly the Air Force General Hospital, PLA) from November 1994 to January 2020 were randomly selected for inclusion in the study. One hundred thirty-nine common hereditary tumor-related genes including *STK11/LKB1* were screened and analyzed for pathogenic germline mutations by high-throughput next-generation sequencing (NGS). The mutation status of the genes and their relationship with clinical phenotypes of PJS were explored.

### *RESULTS*

Twenty of the 24 PJS patients in this group (83.3%) had *STK11/LKB1* gene mutations, 90% of which were pathogenic mutations, and ten had new mutation sites. Pathogenic mutations in exon 7 of *STK11/LKB1* gene were significantly lower than in other exons. Truncation mutations are more common in exons 1 and 4 of *STK11/LKB1*, and their pathogenicity was significantly higher than that of missense mutations. We also found *SLX4* gene mutations in PJS patients.

***CONCLUSION***

PJS has a relatively complicated genetic background. Changes in the sites responsible for coding functional proteins in exon 1 and exon 4 of *STK11/LKB1* may be one of the main causes of PJS. Mutation of the *SLX4* gene may be a cause of genetic heterogeneity in PJS.

**Key Words:** Peutz-Jeghers syndrome; Genotype; Phenotype; STK11; Mutation



**Core Tip:** It is currently believed that Peutz-Jeghers syndrome (PJS) is an autosomal dominant genetic disease predominantly caused by germline mutations in the *STK11/LKB1* gene. No correlation of the PJS genotype and clinical phenotype has been found so far. The correlation of genotype and clinical phenotype and exploration of the internal molecular mechanism of different clinical phenotypes were studied in 24 treated PJS patients with different clinical phenotypes. Peripheral venous blood or normal tissue adjacent to polyps were collected for high-throughput next-generation sequencing (NGS) of 139 hereditary colorectal tumor-related genes including *STK11/LKB1*. A newly discovered likely pathogenic gene (*SLX4*) provided new data explaining the genetic heterogeneity of PJS.

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## INTRODUCTION

It is currently believed that Peutz-Jeghers syndrome (PJS) is an autosomal dominant genetic disease predominantly caused by germline mutations in the *STK11/LKB1* gene. PJS is characterized by multiple hamartoma polyps in the gastrointestinal tract, pigmentation at specific sites, and hereditary tumors[1-4]. Pathogenic mutations of *STK11/LKB1* lead to inactivation of its expression product and loss of inhibition of mammalian target of rapamycin (mTOR) activity, which leads to abnormal activation of the LKB1/mTOR signal pathway and the occurrence of black spots on the skin and gastrointestinal hamartoma polyps[5]. More than 400 different pathogenic *STK11/LKB1* gene mutations are included in the Human Gene Mutation Database (HGMD), most of which are microminiature. Different types of pathogenic mutations may produce different clinical phenotypes, but no correlations of PJS genotype and clinical phenotype has been found so far[6], Not all patients with PJS have detectable mutations in the *STK11/LKB1* gene. What is the genetic basis of clinical phenotypic heterogeneity in PJS? Do PJS patients without *STK11/LKB1* mutations have other pathogenic genes? These are clinical problems that perplex doctors[7,8]. We enrolled 24 patients treated for PJS. Peripheral venous blood and normal tissue adjacent to polyps were collected for high-throughput next-generation sequencing (NGS) of 139 hereditary colorectal tumor-related genes including *STK11/LKB1* to study the correlation between genotype and clinical phenotype of PJS and explore the internal molecular mechanism of the clinical phenotypes.

patients (legal guardians of minors) understood the process and purpose of this study and signed an informed consent form. In the process of sample collection, follow the principles of informed consent in the Declaration of Helsinki, the Universal Declaration of Human Genome and Human Rights, and the Declaration of the Human Genome Ethics Committee on DNA Sampling, Control, and Acquisition. No additional data are available.



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## MATERIALS AND METHODS

### Study participants

Patients with PJS, from 18-70 years of age, met the clinical diagnostic criteria of PJs, had complete clinicopathological data, well preserved specimens, were eligible for inclusion. All participants gave their signed informed consent. Patients who could not provide experimental specimens or did not agree to participate in the study were excluded. Twenty-four PJS patients admitted to the Air Force Medical Center (formerly the Air Force General Hospital) from November 1994 to January 2020 met the above criteria and were enrolled. Their clinical information is shown in Table 1. Twenty-three were inpatients, one was an outpatient, 11 had family histories, and 12 had early onset pigment spots that had appeared when they were younger than 3 years of age. All patients met the PJS diagnostic criteria recommended by the National Comprehensive Cancer Network (NCCN)[9]. The experimental samples included 5 mL peripheral venous blood samples collected from 19 patients into tubes containing EDTA-2Na, and paraffin-embedded normal tissue surgically removed from areas adjacent to polyps in five patients. The study was reviewed and approved by the Ethics Committee of the Air Force Medical Center and the Second Affiliated Hospital of Zhejiang University School of Medicine. All patients or the legal guardians of minors, understood the process and purpose of this study and signed an informed consent form. Sample collection followed the ethical principles of the Declaration of Helsinki, the Universal Declaration of Human Genome and Human Rights, and the Declaration of the Human Genome Ethics Committee on DNA Sampling, Control, and Acquisition.

### Methods

DNA was extracted from peripheral venous blood samples with TGuide Blood Genomic DNA Kits (CHI-TIANGEN) following the manufacturer's instructions. DNA was extracted from paraffin-embedded tissue specimens with QIAamp DNA FFPE micro sample tissue kits (GER-QIAGEN). Nucleic acids were broken into small, random 150-200 bp fragments by ultrasonic fragmentation (Covaris S220) and separated and evaluated with a Tapestation 2200 electrophoresis working platform (Agilent) to check whether the fragments met the requirements for library construction. A standard gene library was constructed using KAPA HyperPlus Kit (Illumina). A panel of 139 common tumor genetic susceptibility genes including colorectal cancer (Table 2) was selected and provided by Genetron Health Co.(Beijing). The specific gene capture probe was hybridized with the library in the environment of a hybridization buffer, and purified by the magnetic bead method. High-throughput NGS was performed with a Novaseq 6000 sequencer (Illumina, United States). Trimmomatic (version 0.33) was used to crop and filter the original data, which was stored in FastQ format, after sequencing. The reads at the end of each pair were aligned with the human reference sequence GRCh37 (hg19) using the BWA-MEM algorithm (BWA version 0.7.10-r789) and the default parameters. The Picard tool (version 1.103 http://broadinstitute.github.io/picard/) was used to delete duplicate readings, and GATK (version 3.1-0-g72492bb) was used to realign the sequences around the known insertion loss at the single sample level and to recalibrate the base quality. Integrative Genomics Viewer version 2.3.34 (https://software.broadinstitute.org/software/igv/) was used to check the mutations in the coding region.

The Chinese (1000 CN), general population (1000 MAF). and dbSNP (https://www.ncbi.nlm.nih.gov/) at 1000 Genome Project (http://ftp.ncbi.nih.gov/) Snip/), ESP6500 AA/EA (NHLBI GO Exome Sequencing Project https://evs gs.washington.edu/EVS/), ExAC MAF (The Exome Aggregation Consortium) and other population databases were searched for the mutation frequency of this gene. The location of genes with a mutation frequency < 0.01 in the HGMD database (HGMD-PUBLIC version 20152) were used for pathogenicity analysis.

The diseases that the variant gene was related to were searched in the OMIM disease database (https://omim.org/) by ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). HGMD https://www.hgmd.cf.ac.uk) retrieved the description of the mutation. SIFT[10] (http://sift.jcvi.org), PolyPhen2[11] (http://genetics.bwh.harvard.edu/pph2), and Mutation Assessor (http://mutationassessor.org) make conservative predictions of amino acid sequences. The results were used to evaluate the pathogenicity of the mutations[12,13].

SPSS 24.0 was used for statistical analysis of the acquired data. Qualitative results were reported as numbers and percentages. The chi-square test or Fisher's exact probability method was used for between-group comparisons. $P < 0.05$ was considered

**Table 1 Clinical characteristics of 24 enrolled Peutz-Jeghers syndrome patients**

| No. | Gender | Specimen | Time since onset of pigment spots (yr) | Early or late onset | Family history (members) | Number of hospitalizations | Number of operations | Stomach and enteroscopy times | Age at initial diagnosis of polyps | Age at first treatment | Polyp pathology | Load of Gastric polyps/Max. diameter (mm) | Load of small intestinal polyps/Max. diameter (mm) | Load of colorectal polyps/Max. diameter (mm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Male | Paraffin section | 20 | Late | No | 2 | 1 | 6 | 20 | 15 | 1 | / | 20/30 | / |
| 2 | Male | Paraffin section | 6 | Late | Yes (mother and sister) | 1 | 2 | 3 | 9 | 9 | 1 | 2/16 | 20/40 | 1/8 |
| 3 | Female | Paraffin section | 4 | Late | No | 2 | 1 | 4 | 9 | 9 | 1 | / | 3/28 | / |
| 4 | Male | Paraffin section | 5 | Late | No | 1 | 2 | 1 | 21 | 21 | 3 | 20/4 | 6/50 | / |
| 5 | Male | Paraffin section | 1 | Early | Yes (mother) | 4 | 2 | 1 | 4 | 4 | 1 | 2/12 | 2/60 | / |
| 6 | Female | Blood | 5 | Late | Yes (father) | 1 | 0 | 1 | 29 | 29 | 1 | / | / | / |
| 7 | Female | Blood | 1 | Early | Yes (father and sister) | 4 | 0 | 11 | 7 | 7 | 1 | 1/8 | 2/30 | 3/40 |
| 8 | Male | Blood | 0 | Early | Yes (father and sister) | 1 | 0 | 1 | 10 | 10 | 1 | / | 10/50 | / |
| 9 | Male | Blood | 6 | Late | Yes (mother and grandmother) | 4 | 1 | 7 | 6 | 7 | 1 | 5/12 | 2/30 | 3/35 |
| 10 | Female | Blood | 2 | Early | No | 1 | 0 | 3 | 7 | 7 | 1 | 2/15 | / | 1/30 |
| 11 | Male | Blood | 3 | Late | No | 1 | 4 | 0 | 22 | 32 | 1 | / | 1/30 | / |
| 12 | Male | Blood | 2 | Early | No | 2 | 1 | 10 | 4 | 4 | 1 | 1/6 | 2/50 | / |
| 13 | Male | Blood | 2 | Early | No | 1 | 2 | 1 | 25 | 24 | 1 | / | 10/20 | / |
| 14 | Female | Blood | 3 | Late | No | 8 | 2 | 8 | 6 | 6 | 1 | 1/10 | 8/80 | 1/20 |
| 15 | Male | Blood | 5 | Late | No | 1 | 2 | 3 | 20 | 19 | 2 | 1/6 | 1/80 | 2/30 |
| 16 | Male | Blood | 1 | Early | Yes (mother) | 3 | 0 | 2 | 10 | 9 | 1 | / | 1/25 | / |
| 17 | Male | Blood | 1 | Early | No | 3 | 1 | 4 | 6 | 6 | 1 | 8/40 | 10/30 | / |
| 18 | Female | Blood | 1 | Early | No | 6 | 2 | 9 | 11 | 10 | 1 | 1/15 | 3/35 | 1/50 |
| 19 | Female | Blood | 3 | Late | Yes (mother) | 2 | 0 | 4 | 15 | 15 | 1 | 1/12 | 2/12 | 1/25 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | Female | Blood | 3 | Late | Yes (father, uncle, and grandmother) | 2 | 2 | 5 | 7 | 7 | 1 | / | 18/50 | / |
| 21 | Female | Blood | 1 | Early | Yes (mother, uncle, and aunt) | 2 | 0 | 4 | 31 | 31 | 1 | / | 10/50 | 10/40 |
| 22 | Female | Blood | 2 | Early | Yes (father and brother) | 1 | 0 | 1 | 6 | 6 | 1 | 10/10 | 8/50 | / |
| 23 | Male | Blood | 5 | Late | No | 1 | 0 | 2 | 11 | 11 | 1 | 1/30 | 5/70 | 1/30 |
| 24 | Male | Blood | 2 | Early | No | 1 | 0 | 4 | 5 | 4 | 1 | 10/15 | / | / |

(1) *STK11* mutation, *SLX4* mutation, other gene mutation groups: 0: None 1: Yes; (2) Early onset: Pigment spots appeared at < 3 years of age; Late onset: Pigment spots appeared at ≥ 3 years of age; (3) Polyp pathology: 1 hamartoma, 2 hamartoma with adenoma, 3 hamartoma with cancer; (4) Polyp load is the number of polyps, the largest diameter unit is mm; and (5) 6 was an outpatient, the results of previous endoscopy are unknown.

statistically significant.

## RESULTS

### *STK11/LKB1* gene detection results and pathogenicity analysis

Twenty of the 24 PJS patients (83.3%) in this group had *STK11/LKB1* gene mutations (Table 3). All were heterozygous and ten were newly discovered mutation sites not included in the dbSNP database. There were eight frameshift mutations, five splice-site mutations, four missense mutations and three nonsense mutations. The mutations occurred in eight of the ten exons in the *STK11/LKB1* gene, mutations in exons 1 and 4 and 4 each in exon 7, two in each exons 5 and 8, and one in exons 2, 3, and 6. Frameshift mutations, splice-site mutations, and nonsense mutations were all related to pathogenicity. Frameshift mutations accounted for 62.5% (5/8) that were clearly pathogenic, and 37.5% (3/8) that might cause disease. Splice-site mutations accounted for 40% (2/5) that are clearly pathogenic, and 60% (3/5) that might cause disease. All three nonsense mutations were clearly pathogenic, and the missense mutations were related to and might cause disease. Sites of unclear clinical significance accounted for 50% (2/4); of the 11 truncated mutations, eight cases were clearly pathogenic and three were likely to cause disease. The pathogenicity of STK11 gene mutations in exon 7 was significantly lower than that of other exons ($P$ = 0.000). Truncation mutations were significantly more pathogenic than missense mutations ($P$ = 0.012). The prediction results of bioinformatics tools for missense mutations are shown in Table 4, and the relevant database records and the pathogenicity judgment of all mutations are shown in Table 5.

**Table 2 Cancer genetic susceptibility 139 gene panel coverage**

| | | | | | |
|---|---|---|---|---|---|
| *AIP* | *CYLD* | *FANCL* | *MLH3* | *PRSS1* | *SMARCA4* |
| *ALK* | *DDB2* | *FANCM* | *MRE11A* | *PTCH1* | *SMARCB1* |
| *APC* | *DICER1* | *FAS* | *MSH2* | *PTCH2* | *SMARCE1* |
| *ATM* | *DIS3L2* | *FH* | *MSH6* | *PTEN* | *SOS1* |
| *ATR* | *EGFR* | *FLCN* | *MTAP* | *PTPN11* | *STAT3* |
| *AXIN2* | *ELANE* | *GALNT12* | *MTUS1* | *RAD50* | *STK11* |
| *BAP1* | *EPCAM* | *GATA2* | *MUTYH* | *RAD51B* | *SUFU* |
| *BARD1* | *ERCC1* | *GEN1* | *NBN* | *RAD51C* | *TERT* |
| *BLM* | *ERCC2* | *GJB2* | *NF1* | *RAD51D* | *TGFBR1* |
| *BMPR1A* | *ERCC3* | *GPC3* | *NF2* | *RB1* | *TMEM127* |
| *BRCA1* | *ERCC4* | *GREM1* | *NSD1* | *RECQL* | *TP53* |
| *BRCA2* | *ERCC5* | *HMBS* | *NTRK1* | *RECQL4* | *TSC1* |
| *BRIP1* | *EXT1* | *HNF1A* | *PALB2* | *RET* | *TSC2* |
| *BUB1B* | *EXT2* | *HOXB13* | *PALLD* | *RHBDF2* | *UROD* |
| *CBL* | *EZH2* | *HRAS* | *PDGFRA* | *RUNX1* | *USHBP1* |
| *CDC73* | *FANCA* | *KIT* | *PHOX2B* | *SBDS* | *VEGFA* |
| *CDH1* | *FANCB* | *LASP1* | *PMS1* | *SDHA* | *VHL* |
| *CDK4* | *FANCC* | *MAX* | *PMS2* | *SDHAF2* | *WRN* |
| *CDKN1B* | *FANCD2* | *MC1R* | *POLD1* | *SDHB* | *WT1* |
| *CDKN1C* | *FANCE* | *MEN1* | *POLE* | *SDHC* | *XPA* |
| *CDKN2A* | *FANCF* | *MET* | *POLH* | *SDHD* | *XPC* |
| *CEBPA* | *FANCG* | *MTTF* | *PPM1D* | *SLX4* | *XRCC2* |
| *CHEK1* | *FANCI* | *MLH1* | *PRKAR1A* | *SMAD4* | *ZMAT3* |
| *CHEK2* | | | | | |

Considering that the type of specimen may impact on the detection rate of *STK11/LKB1* gene mutations, we analyzed the paraffin-embedded tissue and blood samples separately. The detection rate of *STK11/LKB1* mutations in 60 patients with paraffin samples was 60% (3/5), slightly less than the 89.4% (17/19) of the blood samples from 19 patients. The difference in mutation detection rate of this gene in the two types of sample was not statistically different ($P$ = 0.116).

### *SLX4* gene detection results and pathogenicity analysis

*SLX4* gene mutation (Table 6) was detected in 5 PJS patient samples in this group, with a total detection rate of 20.83% (5/24), all of which were heterozygous mutations. The mutation occurred in 4 of 15 exons of *SLX4* gene. Mutation types include: 3 missense mutations, one splice-site mutation, and one non-frameshift mutation. No truncation mutation was found. The *SLX4* gene is a tumor suppressor gene, and there are three newly discovered mutation sites. The prediction results of three cases of missense mutations by bioinformatics tools (Table 7), the collection of relevant databases and the judgment of the pathogenicity of all mutations (Table 8) are as follows.

### Other gene detection results and pathogenicity analysis

A total of 55 mutations of 46 genes other than *STK11/LKB1* and *SLX4* were detected in 21 cases (Table 9), f a detection rate of 87.5% (21/24). Twenty-three of the genes were related to cancer suppression and had 32 different mutation sites. Two mismatch repair *MMR* genes were detected, *MSH2*, *MSH6*. Except for a frameshift mutation (frameshift deletion) in the *BRIP1* gene detected in one patient (No. 18), the rest were missense mutations (Table 10).

**Table 3 Characteristics of *STK11/LKB1* gene mutations**

| No. | Mutation type | dbSNP RS | Mutation site | Amino acid change | Exon | Variant type |
|---|---|---|---|---|---|---|
| 2 | Frameshift | rs372511774 | c.357delC | p.N119Kfs | 2\|10 | SNV |
| 4 | Splice-site variant | rs398123406 | c.921-1G>A | / | 8\|10 | SNP |
| 5 | Frameshift | rs1060499961 | c.131dupA | p.L45Afs | 1\|10 | INS |
| 6 | Missense | / | c.869T>C | p.L290P | 7\|10 | SNP |
| 7 | Nonsense | / | c.658C>T | p.Q220X | 5\|10 | SNP |
| 8 | Frameshift | / | c.548del | p.L183Rfs | 4\|10 | DEL |
| 9 | Splice-site variant | rs398123406 | c.921-1G>C | / | 8\|10 | SNP |
| 10 | Frameshift | / | c.471_472del | p.F157Lfs | 4\|10 | DEL |
| 12 | Frameshift | / | c.180del | p.Y60X | 1\|10 | DEL |
| 13 | Missense | / | c.869T>A | p.L290H | 7\|10 | SNP |
| 14 | Splice-site variant | / | c.598-2A>G | / | 5\|10 | SNP |
| 15 | Missense | rs121913315 | c.580G>A | p.D194N | 4\|10 | SNP |
| 16 | Missense | rs730881978 | c.890G>A | p.R297K | 7\|10 | SNP |
| 17 | Frameshift | / | c.577_578del | p.S193Rfs | 4\|10 | DEL |
| 18 | Splice-site variant | / | c.863-2A>G | / | 7\|10 | SNP |
| 19 | Splice-site variant | rs1555735080 | c.290+1G>T | / | 1\|10 | SNP |
| 20 | Nonsense | / | c.179dup | p.Y60X | 1\|10 | INS |
| 21 | Frameshift | rs587782584 | c.842dup | p.L282Afs | 6\|10 | INS |
| 23 | Frameshift | rs786203886 | c.228dup | p.V77Rfs | 1\|10 | INS |
| 24 | Nonsense | rs730881970 | c.409C>T | p.Q137X | 3\|10 | SNP |

DEL; Deletion; INS: Insertion; SNP: Single nucleotide polymorphism; SNV: Single nucleotide variation.

**Table 4 Prediction of protein function change caused by *STK11/LKB1* mutation**

| No. | PolyPhen | | Mutation Assessor | | SIFT | |
|---|---|---|---|---|---|---|
| | Score | Prediction | Score | Prediction | Score | Prediction |
| 6 | 1 | Probably damaging | 0.98351; 4.21 | High | 0 | Deleterious |
| 13 | 1 | Probably damaging | 0.99415; 4.555 | High | 0 | Deleterious |
| 15 | 1 | Probably damaging | 0.98178; 4.165 | High | 0 | Deleterious |
| 16 | 1 | Probably damaging | 0.98818; 4.34 | High | 0.01 | Deleterious |
| 23 | 0.022 | Benign | 0.56769; 1.78 | Low | 0.26 | Tolerated |

### *STK11/LKB1 genotype-phenotype correlation analysis*

Investigation of the relationship between genotype and family history found that the proportion of patients with truncated mutations was slightly higher in those with a family history than in those without a history (60% *vs* 50%). The proportion of splice-site mutations was lower in those with a family history (20% *vs* 30%), and the proportion of nonsense mutations was higher in patients with a family history (20.0% *vs* 11.1%). The proportions of missense mutations were the same (20% *vs* 20%), and the proportion of frameshift mutations were also equal (40% *vs* 10%). There were no significant difference between-group differences in $P_{truncation\ mutation} = 0.653$, $P_{splice\ site\ mutation} = 0.606$, $P_{nonsense\ mutation} = 0.371$, $P_{missense\ mutation} = 1.000$, and $P_{frameshift\ mutation} = 1.000$.

Evaluation of the relationship between genotype and early onset/late onset found that the proportion of truncated mutations in patients with early onset was higher than that in patients with late onset (72.7% *vs* 33.3%). In patients with early onset, the

**Table 5 *STK11/LKB1* mutation-related databases and pathogenicity analysis**

| No. | cDNA/protein | Disease database | | | Pathogenic judgment |
|---|---|---|---|---|---|
| | | HGMD | ClinVar | OMIM | |
| 2 | p.N119Kfs | / | (1/1) pathogenic | / | Pathogenic |
| 4 | c.921-1G>A | √ | / | PJS | Pathogenic |
| 5 | p.L45Afs | / | / | / | Pathogenic |
| 6 | p.L290P | √ | (1/1) pathogenic | PJS | Clinical significance unknown |
| 7 | p.Q220X | / | (3/3) pathogenic | PJS | Pathogenic |
| 8 | p.L183Rfs | / | / | PJS | Pathogenic |
| 9 | c.921-1G>C | √ | (2/2) pathogenic | PJS | Pathogenic |
| 10 | p.F157Lfs | √ | / | PJS | Likely pathogenic |
| 12 | p.Y60X | √ | √ | PJS | Pathogenic |
| 13 | p.L290H | / | / | PJS | Clinical significance unknown |
| 14 | c.598-2A>G | / | (1/1) pathogenic | PJS | Likely pathogenic |
| 15 | p.D194N | √ | (4/6) likely pathogenic; (2/6) pathogenic | PJS | Likely pathogenic |
| 16 | p.R297K | √ | (1/2) pathogenic; (1/2) unknown | PJS | Likely pathogenic |
| 17 | p.S193Rfs | / | / | PJS | Likely pathogenic |
| 18 | c.863-2A>G | / | (1/1) pathogenic | PJS | Likely pathogenic |
| 19 | c.290+1G>T | Pathogenic | / | PJS | Likely pathogenic |
| 20 | p.Y60X | Pathogenic | (2/2) pathogenic | PJS | Pathogenic |
| 21 | p.L282Afs | Pathogenic | (1/1) pathogenic | PJS | Pathogenic |
| 23 | p.V77Rfs | / | / | PJS | Likely pathogenic |
| 24 | p.Q137X | Pathogenic | (1/1) pathogenic | PJS | Pathogenic |

(4/6) likely pathogenic: A total of six institutions have judged this mutation, four of which are judged as probably pathogenic, the same below. PJS: Peutz-Jeghers syndrome.

**Table 6 Characteristics of *SLX4* gene mutations**

| No. | Mutation type | dbSNP RS | Mutation site | Amino acid changes | Exon | Variant type |
|---|---|---|---|---|---|---|
| 1 | Missense | rs551385115 | c.5072A>G | p.N1691S | 14\|15 | SNP |
| 2 | Splice-site variant | / | c.1683+1G>A | splice | 7\|15 | SNP |
| 3 | Missense | rs774243118 | c.2990C>T | p.P997L | 12\|15 | SNP |
| 18 | Missense | / | c.2425G>C | p.E809Q | 12\|15 | SNP |
| 22 | Non-frameshift | / | c.568_570del | p.P190del | 3\|15 | DEL |

DEL: Deletion; SNP: Single nucleotide polymorphism.

percentages of frameshift mutations (54.5% *vs* 22.2%) and sense mutations (18.2% *vs* 11.1%) were higher than those in late onset patients. The percentages of splice-site mutations (9% *vs* 44.4%) and missense mutations were lower (18.2% *vs* 22.2%). There were no significant between-group differences in $P_{truncation\ mutation} = 0.078$, $P_{frameshift\ mutation} = 0.142$, $P_{nonsense\ mutation} = 0.660$, $P_{splice\ site\ mutation} = 0.069$, $P_{missense\ mutation} = 0.822$.

## DISCUSSION

The *STK11/LKB1* gene located on chromosome 19p13.3 is considered to be a tumor

**Table 7 Prediction of protein function change caused by *SLX4* mutation**

| No. | PolyPhen | | Mutation assessor | | SIFT | |
|---|---|---|---|---|---|---|
| | Score | Prediction | Score | Prediction | Score | Prediction |
| 1 | 0 | Benign | 0.08118; 0 | Neutral | 0.16 | Tolerated |
| 3 | 0.004 | Benign | 0.05510; -0.035 | Neutral | 1 | Tolerated / |
| 18 | 0.341 | Benign | 0.59436; 1.845 | Low | 0.04 | Deleterious |

**Table 8 *SLX4* mutation-related databases and pathogenicity analysis**

| No. | cDNA/Protein | Disease database | | | Pathogenic judgment |
|---|---|---|---|---|---|
| | | HGMD | ClinVar | OMIM | |
| 1 | p.N1691S | / | (1/1)Uncertain Significance | BTB/POZ domain containing 12*SLX4* structure-specific | Clinical significance unknown |
| 2 | c.1683+1G>A | / | / | BTB/POZ domain containing 12\SLX4 structure-specific | Likely pathogenic |
| 3 | p.P997L | / | / | BTB/POZ domain containing 12\SLX4 structure-specific | Clinical significance unknown |
| 18 | p.E809Q | √ | / | BTB (POZ) domain containing 12\SLX4 structure-specific | Clinical significance unknown |
| 22 | p.P190del | / | / | BTB (POZ) domain containing 12\SLX4 structure-specific | Clinical significance unknown |

suppressor gene[14] and is widely expressed in human tissues. Pathogenic mutation of *STK11* can inactivate its expressed product, which results in the loss of its inhibitory effect on the activity of mammalian target of rapamycin (mTOR), leading to the occurrence of skin and mucous membrane black spots and gastrointestinal polyps[5]. Methylation of the *STK11/LKB1* gene promoter has an important role in the process of malignant transformation of gastrointestinal polyps[15]. At present, the comprehensive mutation rate of *STK11/LKB1* gene in PJS patients detected by multiple sequencing methods is about 80%-94%[8,15,16]. The detection rate of *STK11/LKB1* gene mutation in PJS patients in this study was 83.3% (20/24), 90% of which are related to pathogenicity. Analysis of the pathogenicity of all the detected mutation sites included in the Mendelian Inheritance in Man (OMIM) database found that about 90% of the *STK11/LKB1* mutations were related to PJS. Except for the *STK11/LKB1* gene and one case of *SLX4* gene mutation, no other gene mutations related to the disease or the possibility of disease were found.

Research on whether there is a correlation between the PJS genotype and clinical phenotype is ongoing. Although the correlation is currently unclear[6,17], some studies have reported positive results. For example, Forcet *et al*[18] reported that patients often present with only black spots and without gastrointestinal polyps when heterozygous mutations occur in exon 8 of the *STK11* gene. Amos *et al*[19] found that PJS patients with missense mutations had a first episode of polypectomy and appearance of other symptoms significantly later than those with truncated mutations or no detectable mutations. In a study including 116 PJS patients in 52 families, Wang *et al*[20] found that nearly 30% of the mutations occurred in exon 7, and some of those mutations affected the protein Kinase domain XI region, which is associated with 90% of cases with gastrointestinal polyp dysplasia. An analysis of the start region of the *STK11/LKB1* coding sequence by Hearle *et al*[21] found that a change in promoter sequence was unlikely to be the cause of PJS. In this study the time that dark spots first appeared, which is a relatively objective indicator, was the basis of clinical classification, and was used to determine whether there was a correlation between the appearance of the spots and any of the genotypes. Spots that appear in early childhood will be noticed. On the other hand, unless there are obvious clinical symptoms, it is extremely difficult to know about gastrointestinal polyps that appear in early childhood. Also, PJS is an autosomal dominant genetic disease and does not completely follow Mendelian inheritance[6]. In clinical practice, it is often found that neither parent has a family history but their child has the disease. This is difficult to fully explain if the disease is caused by a single gene. Therefore, whether the patient has a family history was also included in the basis of clinical classification.

This study did not found that patients with different clinical phenotypes (early onset/late onset and with or without a family history) had statistically significant differences in their *STK11/LKB1* gene mutations and loci. However, we found that the

**Table 9 Other gene mutations and inclusion in relevant database**

| No. | Gene | Type | Mutation site | Amino acid changes | Exon | Disease database | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | HGMD | ClinVar | OMIM |
| 1 | *BARD1* | TSG | c.556A>G | p.S186G | 4\|11 | / | (6/6)Uncertain Significance | / |
| | *EGFR* | / | c.61G>A | p.A21T | 1\|28 | / | / | Epidermal growth factor receptor |
| 2 | *GEN1* | / | c.181T>A | p.S61T | 3\|14 | / | / | Gen endonuclease homolog 1 |
| | *BRCA1* | TSG | c.2387C>T | p.T796I | 10\|23 | / | (8/8)Uncertain Significance | / |
| 4 | *NTRK1* | / | c.1604A>G | p.E535G | 13\|17 | / | / | / |
| | *PDGFRA* | / | c.1423G>A | p.E475K | 10\|23 | / | / | / |
| | *TSC2* | TSG | c.521C>T | p.S174L | 6\|42 | / | (2/2)Uncertain Significance | / |
| | *MSH6* | / | c.1063G>A | p.G355S | 4\|10 | | (4/7)Uncertain Significance(3/7)likely benign | / |
| 5 | *EGFR* | / | c.3040G>A | p.D1014N | 25\|28 | / | / | Epidermal growth factor receptor |
| | *MTUS1* | TSG | c.2282G>A | p.S761N | 3\|15 | / | / | Mitochondrial tumor suppressor 1 |
| | *PTCH1* | TSG | c.2222C>T | p.A741V | 14\|24 | / | (3/4)benign, (1/4)likely benign | / |
| 6 | *SDHA* | TSG | c.715A>G | p.I239V | 6\|15 | √ | (2/2)Uncertain significance | / |
| | *MTUS1* | TSG | c.1866C>G | p.N622K | 2\|15 | √ | √ | Mitochondrial tumor suppressor 1 |
| 7 | *RECQL4* | / | c.1048A>G | p.R350G | 5\|21 | / | (1/1)Uncertain Significance | / |
| | *RECQL4* | / | c.236G>A | p.G79E | 4\|21 | / | / | / |
| 8 | *ATM* | TSG | c.6503C>T | p.S2168L | 45\|63 | / | (7/7)Uncertain Significance | Ataxia telangiectasia mutated |
| 10 | *TSC2* | TSG | c.3475C>T | p.R1159W | 30\|42 | / | (2/4)benign, (2/4)likely benign | / |
| | *FANCG* | TSG | c.458C>G | p.A153G | 4\|14 | / | (1/1)Uncertain Significance | / |
| 11 | *SBDS* | / | c.98A>G | p.K33R | 1\|5 | / | / | / |
| 12 | *VHL* | TSG | c.134C>T | p.P45L | 1\|3 | / | / | Von Hippel-Lindau syndrome |
| | *FANCA* | / | c.3031C>T | p.R1011C | 31\|43 | / | (1/1)likely benign | / |
| | *TP53* | TSG | c.620A>G | p.D207G | 6\|11 | √ | / | / |
| 13 | *FANCA* | / | c.2944A>G | p.T982A | 30\|43 | / | (2/2)Uncertain Significance | / |
| 14 | *PALLD* | / | c.1011C>A | p.D337E | 3\|21 | / | / | / |
| | *MLH3* | TSG | c.1519A>G | p.M507V | 2\|13 | / | (1/1)Uncertain Significance | Mutl (E. Coli) homolog 3 |
| | *SMARCA4* | TSG | c.3791C>T | p.T1264M | 28\|36 | / | (3/3)Uncertain Significance | / |
| | *NF1* | TSG | c.3940T>C | p.W1314R | 29\|58 | / | (1/1)Uncertain Significance | / |
| 15 | *PTCH1* | TSG | c.2222C>T | p.A741V | 14\|24 | / | (1/1)likely benign | / |
| | *GALNT12* | / | c.148C>A | p.P50T | 1\|10 | / | / | / |
| 16 | *ATR* | TSG | c.325C>T | p.R109W | 4\|47 | / | (1/1)Uncertain Significance | Ataxia telangiectasia and Rad3 related |
| | *VEGFA* | TSG | c.1039G>A | p.V347I | 6\|8 | / | / | Vascular endothelial growth factor |
| | *DIS3L2* | / | c.1642G>A | p.A548T | 13\|21 | / | / | / |
| 17 | *TSC1* | TSG | c.2693C>G | p.T898S | 21\|23 | √ | (3/5)likely benign, (1/5)benign, (1/5)Uncertain significance | / |
| 18 | *PTCH1* | TSG | c.109G>T | p.G37W | 1\|24 | √ | (1/1)Uncertain Significance | / |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *BRIP1* | / | c.3072del | p.S1025Hfs | 20\|20 | √ | (1/2)likely pathogenic, (1/2)Uncertain significance | / |
| | *WRN* | / | c.3778G>A | p.A1260T | 32\|35 | / | (2/2)Uncertain significance | werner syndrome |
| | *RECQL* | / | c.166G>A | p.G56R | 4\|16 | / | / | / |
| 19 | *BARD1* | TSG | c.1148T>G | p.M383R | 4\|11 | / | / | / |
| | *USHBP1* | / | c.1358C>T | p.P453L | 9\|13 | / | / | / |
| | *APC* | TSG | c.2882A>G | p.N961S | 16\|16 | / | (1/1)Uncertain Significance | Adenomatosis polyposis coli |
| 20 | *DICER1* | TSG | c.2113A>G | p.I705V | 13\|27 | / | / | Multinodular goiter |
| | *FANCM* | / | c.2762G>A | p.C921Y | 14\|23 | / | / | / |
| | *APC* | TSG | c.5257G>C | p.A1753P | 16\|16 | / | (3/3)Uncertain Significance | Adenomatosis polyposis coli |
| | *NSD1* | / | c.5493T>G | p.D1831E | 16\|23 | / | / | Sotos syndrome |
| | *SDHA* | TSG | c.739A>G | p.I247V | 6\|15 | / | (4/4)Uncertain Significance | / |
| | *MTUS1* | TSG | c.908A>G | p.N303S | 2\|15 | / | / | Mitochondrial tumor suppressor 1 |
| 22 | *EXT2* | TSG | c.896G>A | p.R299H | 5\|14 | √ | (1/2)likely benign, (1/2)uncategorized | / |
| | *ATM* | TSG | c.1555G>A | p.V519I | 10\|63 | √ | (3/3)Uncertain Significance | Ataxia telangiectasia mutated |
| | *BRCA2* | TSG | c.1568A>G | p.H523R | 10\|27 | √ | (1/12)benign, (9/12)likely benign, (2/12)Uncertain Significance | Fanconi anemia |
| | *TP53* | TSG | c.214C>G | p.P72A | 4\|11 | √ | (5/5)Uncertain Significance | / |
| 23 | *FLCN* | TSG | c.1366G>C | p.D456H | 12\|14 | / | / | |
| | *MSH2* | TSG | c.1789G>A | p.D597N | 12\|16 | / | (1/1)Uncertain Significance | Colon cancer, nonpolyposis type 1 |
| | *KIT* | / | c.2263G>A | p.A755T | 16\|21 | / | (1/2)Uncertain Significance,(1/2)uncategorized | Piebald trait |
| 24 | *BAP1* | TSG | c.1154G>A | p.R385Q | 12\|17 | / | (2/2)Uncertain Significance | / |
| | *TSC2* | TSG | c.1609C>T | p.R537C | 16\|42 | √ | (1/5)benign, (2/5)likely benign; (1/5)Uncertain Significance; (1/5)uncategorized | / |

HGMD: Human Gene Mutation Database; OMIM: Online Mendelian Inheritance in Man; TSG: Tumor suppressor gene.

most truncation mutations of the *STK11/LKB1* gene mostly occurred in exons 1 and 4, most missense mutations occurred in exon 7, and that truncation mutations were significantly more pathogenic than missense mutations. The results indicate that changes in the sites encoding functional proteins in exon regions 1 and 4 may be among the main causes of PJS. Also, the percentage of *STK11/LKB1* truncation mutations in patients with early onset PJS was higher than that in patients with late onset PJS, and the between-group difference in the percentage of missense mutations was not significant. Because the evidence of a correlation with missense mutations was not strong, it suggests that early onset PJS is more likely to be caused by pathogenic mutations in *STK11/LKB1*, while late onset disease is likely to be clinically heterogeneous. The study results also suggest that analysis of the age of appearance of dark spots in a large sample of PJS patients would yield some interesting findings.

For the first time, we detected more concentrated mutations in the *SLX4* gene in PJS patients. The *SLX4* (*FANCP*) gene is a tumor suppressor gene located on chromosome 16p13.3[21]. It serves as a key scaffold element for the assembly of multiprotein complexes containing enzymes involved in DNA maintenance and repair[22] and has low to moderate expression in all adult and fetal tissues and specific adult brain regions[23]. It has been reported that[24] truncated mutations in the *SLX4* gene were detected in families with Fanconi anemia, and it was determined that *SLX4* mutations are clearly related to one of the subtypes of the disease. Fanconi anemia is a rare autosomal recessive genetic disease[25]. In addition to blood system-related manifestations, the clinical manifestations of FA include multiple congenital malformations, brown pigmentation of the skin, and tumor susceptibility[26]. There are many similarities with PJS, mutations in the *SLX4* gene have been detected in patients with PJS in previous studies, the first of which was found in this group. *SLX4* is considered

**Table 10 Prediction of protein function changes caused by other gene mutations**

| Gene | SIFT | | PolyPhen | | Mutation Assessor | |
|---|---|---|---|---|---|---|
| | Score | Prediction | Score | Prediction | Score | Prediction |
| *BARD1* | 0 | Deleterious | 0.144 | Benign | 0.66939; 2.045 | Medium |
| *EGFR* | 0.4 | Tolerated | 0.956 | Probably damaging | 0.33485; 1.01 | Low |
| *GEN1* | 0 | Deleterious | 0.999 | Probably damaging | 0.34521; 1.04 | Low |
| *BRCA1* | 0.02 | Deleterious | 0.775 | Probably damaging | 0.78223; 2.4 | Medium |
| *NTRK1* | 0.01 | Deleterious | 0.639 | Probably damaging | 0.02685; -0.53 | Neutral |
| *PDGFRA* | 0.1 | Tolerated | 0.05 | Benign | 0.38838; 1.175 | Low |
| *TSC2* | 0.15 | Tolerated | 0.327 | Benign | 0.57536; 1.79 | Low |
| *MSH6* | 0.45 | Tolerated | 0.176 | Benign | 0.08118; 0 | Neutral |
| *EGFR* | 0 | Deleterious | 0.814 | Possibly damaging | 0.83953; 2.67 | Medium |
| *MTUS1* | 0.09 | Tolerated | 0.044 | Benign | 0.27053; 0.805 | Low |
| *PTCH1* | 0 | Deleterious | 0.7 | Possibly damaging | 0.88377; 2.95 | Medium |
| *SDHA* | 0.01 | Deleterious low confidence | 0.078 | Benign | 0.49699; 1.58 | Low |
| *MTUS1* | 0.01 | Deleterious | 0.096 | Benign | 0.29908; 0.895 | Low |
| *RECQL4* | / | / | / | / | / | / |
| *RECQL4* | / | / | / | / | / | / |
| *ATM* | 0 | Deleterious | 0.294 | Benign | 0.67953; 2.075 | Medium |
| *TSC2* | 0.01 | Deleterious | 0.226 | Benign | 0.08118; 0 | Neutral |
| *FANCG* | 0.03 | Deleterious | 0.018 | Benign | 0.14661; 0.345 | Neutral |
| *SBDS* | 0.12 | Tolerated | 0.051 | Benign | 0.71920; 2.185 | Medium |
| *VHL* | 0.06 | Tolerated | 0.012 | Benign | 0.19112; 0.55 | Neutral |
| *FANCA* | 0.24 | Tolerated | 0 | Benign | 0.02315; -0.6 | Neutral |
| *TP53* | 0.03 | Deleterious | 0.386 | Benign | 0.45228; 1.405 | Low |
| *FANCA* | 0.79 | Tolerated | 0.007 | Benign | 0.52573; 1.65 | Low |
| *PALLD* | 0.7 | Tolerated | 0.159 | Benign | 0.00602; -1.34 | Neutral |
| *MLH3* | 0.47 | Tolerated | 0 | Benign | 0.55103; 1.725 | Low |
| *SMARCA4* | 0.05 | Deleterious | 0.007 | Benign | 0.29908; 0.895 | Low |
| *NF1* | 0.62 | Tolerated | 0.015 | Benign | 0.08118; 0 | Neutral |
| *PTCH1* | 0 | Deleterious | 0.626 | Possibly damaging | 0.88377; 2.95 | Medium |
| *GALNT12* | 0.11 | Tolerated | 0.007 | Benign | 0.51422; 1.61 | Low |
| *ATR* | 0 | Deleterious | 0.998 | Probably damaging | 0.65975; 2.015 | Medium |
| *VEGFA* | 0.25 | Tolerated low confidence | 0.695 | Probably damaging | 0.08118; 0 | Neutral |
| *DIS3L2* | 0.05 | Tolerated | 0.996 | Probably damaging | 0.87328; 2.875 | Medium |
| *TSC1* | / | / | | / | 0.00621; -1.32 | Neutral |
| *PTCH1* | 0.03 | Deleterious low confidence | 0.259 | Benign | 0.36672; 1.1 | Low |
| *BRIP1* | / | / | / | / | / | / |
| *WRN* | 0.59 | Tolerated | 0.164 | Benign | 0.70595; 2.14 | Medium |
| *RECQL* | 0.5 | Tolerated | 0.005 | Benign | 0.41079; 1.255 | Low |
| *BARD1* | 0.4 | Tolerated | 0 | Benign | 0.08118; 0 | Neutral |
| *USHBP1* | 0.05 | Tolerated | 0.521 | Possibly damaging | 0.56769; 1.78 | Low |
| *APC* | 0.16 | Tolerated | 0.82 | Possibly damaging | 0.46157; 1.445 | Low |

| | | | | | | |
|---|---|---|---|---|---|---|
| *DICER1* | 0.29 | Tolerated | 0.664 | Possibly damaging | 0.34521; 1.04 | Low |
| *FANCM* | 1 | Tolerated | 0 | Benign | 0.40543; 1.245 | Low |
| *APC* | 0.57 | Tolerated low confidence | 0.003 | Benign | 0.14661; 0.345 | Neutral |
| *NSD1* | 0.03 | Deleterious | 0.684 | Possibly damaging | 0.66939; 2.045 | Medium |
| *SDHA* | 0.02 | Deleterious low confidence | 0.02 | Benign | 0.20574; 0.59 | Neutral |
| *MTUS1* | 0.87 | Tolerated | 0 | Benign | 0.12746; 0.255 | Neutral |
| *EXT2* | 0.03 | Deleterious | 0.993 | Possibly damaging | 0.82323; 2.585 | Medium |
| *ATM* | 0.58 | Tolerated | 0.007 | Benign | 0.56769; 1.78 | Low |
| *BRCA2* | 0.09 | Tolerated | 0.003 | Benign | 0.08118; 0 | Neutral |
| *TP53* | 0.94 | Tolerated | 0 | Benign | 0.03608; -0.345 | Neutral |
| *FLCN* | 0.03 | Deleterious | 0 | Benign | 0.47716; 1.5 | Low |
| *MSH2* | 0.25 | Tolerated | 0.023 | Benign | 0.39692;1.235 | Low |
| *KIT* | 0.15 | Tolerated | 0.472 | Possibly damaging | 0.03608; -0.345 | Neutral |
| *BAP1* | 0 | Deleterious low confidence | 0.968 | Possibly damaging | 0.59436; 1.845 | Low |
| *TSC2* | 0.02 | Deleterious | 0.446 | Possibly damaging | 0.75777; 2.31 | Medium |

to be an important regulator of DNA repair. Studies have shown that repairing specific types of DNA damage requires *SLX4* and other endonucleases to participate together [22]. At present, it is believed that[27-29] the loss of DNA MMR genes causes the accumulation of mismatches in the process of DNA replication, resulting in the occurrence of microsatellite instability and partial junctions. Colorectal cancer has obvious genetic characteristics. We also detected mutations in some MMR genes ( *MSH2* and *MSH6*) in PJS, and the role of *SLX4* gene is highly similar to that. Perhaps the mutation of the *SLX4* gene may explain the genetic heterogeneity of PJS to some extent.

## CONCLUSION

In conclusion, we discovered a series of new gene mutation sites, analyzed their pathogenicity, and enriched the mutation spectrum of PJS pathogenic genes. And through the summary of the clinical phenotypes with different *STK11* genotypes, to explore whether they are related, and get some tendentious research results. The detection of *SLX4* gene mutations in patients with PJS was reported for the first time. The relationship between *SLX4* gene mutations and the occurrence of PJS is still unclear, but may help to explain the genetic heterogeneity of PJS.

## ARTICLE HIGHLIGHTS

### *Research background*

Different types of pathogenic mutations may produce different clinical phenotypes, but no exact correlation between Peutz-Jeghers syndrome (PJS) genotype and clinical phenotype has been found so far. So it is necessary to study the correlation between genotype and clinical phenotype of PJS, and explore the internal molecular mechanism of different clinical phenotypes.

### *Research motivation*

The authors included 24 cases of treated PJS cases as study participants, collected peripheral venous blood or normal tissue adjacent to polyps for high-throughput next-generation sequencing (NGS) of 139 hereditary colorectal tumor-related genes including *STK11/LKB1* to study the correlation between genotype and clinical phenotype of PJS.

### Research objectives

To investigate the correlation between the genotype and clinical phenotype of PJS.

### Research methods

Twenty-four patients with PJS were randomly selected for study inclusion. A total of 139 common hereditary tumor-related genes including *STK11/LKB1* were screened and analyzed for pathogenic germline mutations by high-throughput next-generation sequencing (NGS), and the pathogenicity of these mutations was evaluated.

### Research results

*STK11/LKB1* gene mutations were identified in 20 PJS patients, 90% of which were pathogenic mutations. 10 cases had new mutation sites. Pathogenic mutations were significantly less frequent in exon 7 of the *STK11/LKB1* gene than in other exons. Truncation mutations were more common in exons 1 and 4, and their pathogenicity was significantly higher than that of missense mutations. We also identified *SLX4* gene mutations in PJS patients.

### Research conclusions

PJS has a relatively complicated genetic background. Changes in the sites responsible for coding functional proteins in exon 1 and exon 4 of *STK11/LKB1* may be one of the main causes of PJS. Mutation of the *SLX4* gene may help to explain the genetic heterogeneity of PJS.

### Research perspectives

Exploration of the relationships of clinical phenotypes with different *STK11* genotypes, may help to interpret some controversial research results. The detection of *SLX4* gene mutations in patients with PJS was reported for the first time.

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