

World Journal of *Gastroenterology*

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OPINION REVIEW

- 1847 Malignant gastric outlet obstruction: Which is the best therapeutic option?
Troncone E, Fugazza A, Cappello A, Del Vecchio Blanco G, Monteleone G, Repici A, Teoh AYB, Anderloni A

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- 1861 Macrophages in metabolic associated fatty liver disease
Alharthi J, Latchoumanin O, George J, Eslam M

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- 1879 Regulation of macrophage activation in the liver after acute injury: Role of the fibrinolytic system
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- 1888 Sequencing of systemic treatment for hepatocellular carcinoma: Second line competitors
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- 1901 Therapeutic advances in non-alcoholic fatty liver disease: A microbiota-centered view
Chen HT, Huang HL, Li YQ, Xu HM, Zhou YJ

ORIGINAL ARTICLE**Basic Study**

- 1912 Interleukin-6 compared to the other Th17/Treg related cytokines in inflammatory bowel disease and colorectal cancer
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- 1926 Mutation analysis of related genes in hamartoma polyp tissue of Peutz-Jeghers syndrome
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- 1938 Iron metabolism imbalance at the time of listing increases overall and infectious mortality after liver transplantation
Fallet E, Rayar M, Landrieux A, Camus C, Houssel-Debry P, Jezequel C, Legros L, Uguen T, Ropert-Bouchet M, Boudjema K, Guyader D, Bardou-Jacquet E

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- 1950 Effectiveness of very low-volume preparation for colonoscopy: A prospective, multicenter observational study
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- 1962** Retrograde inspection *vs* standard forward view for the detection of colorectal adenomas during colonoscopy: A back-to-back randomized clinical trial
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CASE REPORT

- 1971** Severe steroid refractory gastritis induced by Nivolumab: A case report
Vindum HH, Agnholt JS, Nielsen AWM, Nielsen MB, Schmidt H
- 1979** Efficacy of bevacizumab-containing chemotherapy in metastatic colorectal cancer and *CXCL5* expression: Six case reports
Novillo A, Gaibar M, Romero-Lorca A, Gilsanz MF, Beltrán L, Galán M, Antón B, Malón D, Moreno A, Fernández-Santander A

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Basic Study

Mutation analysis of related genes in hamartoma polyp tissue of Peutz-Jeghers syndrome

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Abstract

BACKGROUND

Peutz-Jeghers syndrome (PJS) is a rare disease with clinical manifestations of pigmented spots on the lips, mucous membranes and extremities, scattered gastrointestinal polyps, and susceptibility to tumors. The clinical heterogeneity of PJS is obvious, and the relationship between clinical phenotype and genotype is still unclear.

AIM

To investigate the mutation status of hereditary colorectal tumor-associated genes in hamartoma polyp tissue of PJS patients and discuss its relationship with the clinicopathological data of PJS.

METHODS

Twenty patients with PJS were randomly selected for this study and were treated in the Air Force Medical Center (former Air Force General Hospital) PLA between 2008 and 2017. Their hamartoma polyp tissues were used for *APC*, *AXIN2*, *BMPR1A*, *EPCAM*, *MLH1*, *MLH3*, *MSH2*, *MSH6*, *MUTYH*, *PMS1*, *PMS2*, *PTEN*, *SMAD4*, and *LKB1/STK11* gene sequencing using next-generation sequencing technology. The correlations between the sequencing results and clinical pathological data of PJS were analyzed.

RESULTS

Fourteen types of *LKB1/STK11* mutations were detected in 16 cases (80.0%), of which 8 new mutations were found (3 types of frameshift deletion mutations: c.243delG, c.363_364delGA, and c.722delC; 2 types of frameshift insertions: c.144_145insGCAAG, and c.454_455insC; 3 types of splice site mutations: c.464+1G>T, c.464+1G>A, and c.598-1G>A); 9 cases (45.0%) were found to have

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18 types of heterozygous mutations in the remaining 13 genes except *LKB1/STK11*. Of these, *MSH2*: c.792+1G>A, *MSH6*: c.3689C>G, c.4001+13C>CTTAC, *PMS1*: c.46C>t, and c.922G>A were new mutations.

CONCLUSION

The genetic mutations in hamartoma polyp tissue of PJS are complex and diverse. Moreover, other gene mutations in PJS hamartoma polyp tissue were observed, with the exception of *LKB1/STK11* gene, especially the *DNA mismatch repair gene (MMR)*. Colorectal hamartoma polyps with *LKB1/STK11* mutations were larger in diameter than those with other gene mutations.

Key words: Peutz-Jeghers syndrome; *STK11* gene; *LKB1* gene; Sequencing; Genetic analysis

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Core tip: Peutz-Jeghers syndrome (PJS) is currently considered an autosomal dominant inherited disease caused by germline mutations of the *LKB1/STK11* gene. Gastrointestinal hamartoma polyps are one of the most common clinical manifestations. In order to investigate the mutation status of these familiar genetically-related genes in PJS hamartoma polyp tissues, high-throughput sequencing was used to analyze the mutations of related genes in PJS hamartoma polyps. In addition, the relationships between the mutation status and the clinical pathological data of PJS are discussed.

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INTRODUCTION

Peutz-Jeghers syndrome (PJS) is a rare autosomal dominant inherited disease. The main manifestation of PJS is hamartoma polyps throughout the gastrointestinal tract^[1,2]. It is believed^[3-5] that germline mutations of the tumor suppressor gene *LKB1/STK11* are involved in the etiology of PJS. The encoded product of *LKB1/STK11* gene is a serine/threonine protein kinase which is widely distributed in various tissues^[6,7] and plays an important role in regulating cellular energy metabolism, chromatin remodeling, DNA damage response, cell cycle arrest, p53-mediated apoptosis, as well as cell polarization^[8-10]. Although PJS is a rare clinical disease, these hamartoma polyps can cause serious clinical damage and obvious heterogeneity of clinical phenotypes. Therefore, it is necessary to study the mutations of *LKB1/STK11* gene and other hereditary colorectal tumor-associated genes in PJS hamartoma polyp tissue to investigate the correlation between genotype and phenotype. Twenty patients with PJS were randomly selected for this study, and were treated in the Air Force Medical Center (former Air Force General Hospital) PLA between 2008 and 2017. Fourteen genetically-related genes (*APC*, *AXIN2*, *BMPR1A*, *EPCAM*, *MLH1*, *MLH3*, *MSH2*, *MSH6*, *MUTYH*, *PMS1*, *PMS2*, *PTEN*, *SMAD4*, *LKB1/STK11*) were sequenced in hamartoma polyp tissue from these patients using next-generation sequencing technology to determine the mutation status of these familiar genetically-related genes in PJS hamartoma polyp tissues, and examine the relationship between the mutation status of these genes and the clinical pathological data of PJS.

MATERIALS AND METHODS

Clinical data

Twenty patients with PJS were randomly selected for this study, and were treated in the Air Force Medical Center (former Air Force General Hospital) PLA between 2008 and 2017. All patients met the diagnostic criteria for PJS recommended by the National Comprehensive Cancer Network^[11], and complied with the guidelines of the Declaration of Helsinki. The guardians of children and adult patients were informed

of the purpose of the study, and signed an informed consent form. Their complete clinicopathological data were recorded, and hamartoma polyp tissue samples were obtained and preserved, excluding cancerous polyps (Table 1).

Experimental method

The genomic DNA was extracted from PJS polyp tissue using the QIAamp DNA FFPE Tissue Kit microsample genomic DNA extraction kit, and the experiment was performed according to the kit instructions (QIAamp Tissue DNA FFPE Tissue Kit, QIAGEN, QIAGEN Strasse 1407124 Hilden, Germany).

A normalized cDNA library was built using Ion AmpliSeq Library Kit 2.0 according to the manufacturer's instructions. Two types of Ion AmpliSeq custom panels: IAD72340_182_pool 1 and IAD72340_182_pool 2, were used as multiplex PCR primers, which covered all exons and exon-intron junctions of 14 common hereditary colorectal tumor-associated genes (*APC*, *AXIN2*, *BMPR1A*, *EPCAM*, *MLH1*, *MLH3*, *MSH2*, *MSH6*, *MUTYH*, *PMS1*, *PMS2*, *PTEN*, *SMAD4*, *LKB1/STK11*). After amplification, the paramagnetic particle method (AMPure XP Reagent, Beckman, United States) was used to purify the library. The library was quantitatively detected using fluorescence quantitative PCR (ViiA 7 Dx, Life Technologies Holdings PTE Ltd Block, Singapore city, Singapore). Template preparation (Ion OneTouch2) and template enrichment (Ion OneTouch ES) was then performed using an automated template preparation instrument (Ion OneTouch™ 2 system). High-throughput sequencing was performed using sequencer Ion PGM (Life Technologies).

Quality control sequencing data with a target capture rate > 75%, coverage uniformity > 80%, and average sequencing depth > 150× were used as parameters, and the sequencing results were analyzed using Torrent Suite software (Life Technologies; v5.0.4) and compared using the hg19 Human reference genome. The detected gene mutations were annotated with Ion Reporter software (<https://ionreporter.lifetechnologies.com/ir/secure/home.html>) and ANNOVAR package software (<http://wannovar.wglab.org/>).

Candidate verification sites were screened according to the mutation frequency. The dbSNP database (<http://www.ncbi.nlm.nih.gov/projects/SNP/>), 1000 Genomes Project (<http://ftp.ncbi.nih.gov/>) and the genome Aggregation Database (gnomAD, <http://gnomad.broadinstitute.org/>) were used in the population frequency database. Suspect or clear pathogenic sites included in HGMD (version 2017.03, <http://www.hgmd.cf.ac.uk/ac/index.php>) and database frequency < 0.01, and between 0.01 and 0.05 were retained for verification.

Prime3 online software (<http://bioinfo.ut.ee/primer3/>) was used to design PCR primers for candidate verification sites^[12]. The designed primers were synthesized by Xi'an Qingke Biological Company. The primers were detected and purified after amplification, and were sequenced using the AB 3500xl Dx automatic DNA sequencer (Xi'an Qingke Biological Co., Ltd.). The results verified the preliminary screening of candidate sites.

Protein functional prediction of mutant genes using software Polymorphism Phenotyping v2 (PolyPhen-2, <http://genetics.bwh.harvard.edu/pph2/index.shtml>), MutationTaster (<http://www.mutationtaster.org/>), Functional Analysis through Hidden Markov Models (FATHMM, <http://fathmm.biocompute.org.uk/index.html>) and Mendelian Clinically Applicable Pathogenicity (M-CAP, <http://bejerano.stanford.edu/MCAP/>) for primary screening candidate sites verified by first-generation sequencing, and software GERP++ (<http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html>) and PhyloP (<http://compugen.bscb.cornell.edu/phast>) were used to make conservative predictions of amino acid evolution. Protein models were built using SWISS MODEL (<https://www.swissmodel.expasy.org/>) online software.

Statistical analyses

Statistical analysis of the data was performed using the SPSS 24.0 software package. The normal distribution measurement data are expressed as the mean ± SD, and the non-normal distribution data are described as the median (interquartile range Q). The number of statistical data and the composition ratio were compared. The χ^2 test or Fisher's exact probability method was used to compare the composition of the groups. In the quantitative data, the time of occurrence of dark spots, the interval between the appearance of dark spots and abdominal symptoms, the age at initial diagnosis and the maximum diameter of polyps, *etc.* were determined. The inter-group comparison of the state distribution data was performed using the Mann-Whitney *U* test or Kruskal-Wallis *H* test. *P* < 0.05 was considered statistically significant.

Table 1 Clinicopathological data of enrolled patients with Peutz-Jeghers syndrome

Case No.	Onset time of pigment spots (yr)	Gender	Family history or not (detail)	Load of gastric polyps	Maximum diameter of gastric polyps (mm)	Load of duodenal and small intestinal polyps	Maximum diameter of duodenal and small intestinal polyps (mm)	Load of colorectal polyps	Maximum diameter of colorectal polyps (mm)	Number of hospitalization times	Number of operation times	Number of intervention times
1	0	Male	Yes (Father)	0	0	1-10	30	Unknown	Unknown	1	1	3
2	0	Male	No	1-10	10	31-40	80	11-20	70	3	1	4
3	7	Female	Yes (Father)	1-10	6	21-30	25	Unknown	Unknown	1	1	4
4	2	Male	No	11-20	17	1-10	25	1-10	40	1	1	4
5	10	Male	Yes (Son)	1-10	5	51-60	50	41-50	25	5	3	12
6	1	Male	Yes (Mother)	1-10	5	1-10	35	1-10	35	2	1	3
7	1	Male	No	1-10	23	1-10	50	Unknown	Unknown	1	0	2
8	1	Male	Yes (Grandmother and mother)	0	0	Unknown	Unknown	51-60	70	1	4	20
9	7	Male	Yes (Father)	0	0	11-20	60	21-30	12	1	1	9
10	13	Female	No	0	0	1-10	40	1-10	10	3	4	9
11	2	Male	No	1-10	8	1-10	25	1-10	6	2	0	4
12	0	Female	No	1-10	15	41-50	60	11-20	50	2	4	9
13	5	Female	Yes (Father and brother)	21-30	15	41-50	60	21-30	60	4	2	9
14	18	Female	No	21-30	5	11-20	30	1-10	6	2	1	5
15	0.8	Female	Yes (Father)	1-10	6	1-10	6	0	0	2	0	4
16	2	Male	Yes (Father)	21-30	20	31-40	45	51-60	45	2	7	21
17	4	Female	Yes (Son)	Unknown	Unknown	21-30	30	1-10	30	1	1	5
18	0	Male	No	1-10	15	Unknown	Unknown	1-10	20	1	0	2
19	5	Female	No	0	0	11-20	20	Unknown	Unknown	1	2	7
20	4	Female	Yes (Sister)	1-10	50	21-30	50	Unknown	Unknown	1	0	7

RESULTS

Mutations of LKB1/STK11 gene

In this patient group, *LKB1/STK11* gene mutations were detected in 16 of 20 cases, with 14 types of mutations, of which 8 new mutations were detected. According to the prediction of Mutationtaster software, 8 types of protein truncation mutations were found in 10 cases (2 types of nonsense mutations detected in 3 cases, 6 types of frameshift mutations in 7 cases). Among them, the frameshift mutations can cause truncation protein mutations (Table 2). These mutations can change protein function and the prediction of amino acid evolution conservation is shown in Table 3.

Mutation of other 13 genes with the exception of LKB1/STK11

In this patient group, 18 types of gene mutations were detected in 9 of 20 cases, all of which were heterozygous mutations (Table 4). The prediction of protein function change and amino acid evolution conservation caused by the mutations are shown in Table 5 and Table 6. It is less likely that the *PMS2* mutation in patient No. 3 and 4 and

Table 2 Mutation status of *LKB1/STK11* gene

Case No.	Allele	Mutation type	Exon/intron	Amino acid change	Base change	New mutation
1	Heterozygosis	Missense	4	p.L167R	c.500T>G	No
2	Heterozygosis	Nonsense	1	p.K84*	c.250A>T	No
3	Heterozygosis	Frameshift deletion	5	p.A241Vfs*46	c.722delC	Yes
4	Homozygous	Frameshift insertion	3	p.Q152Pfs*11	c.454_455insC	Yes
5	Heterozygosis	Frameshift insertion	1	p.Y49Afs*4	c.144_145insGCAAAG	Yes
6	Heterozygosis	Missense	5	p.S240W	c.719C>G	No
7	Heterozygosis	Frameshift deletion	1	p.K82Rfs*14	c.243delG	Yes
8	Heterozygosis	Cleavage site	5-6 ¹	/	c.734+1G>A	Yes
10	Heterozygosis	Cleavage site	3-4 ¹	/	c.464+1G>T	Yes
13	Homozygous	Frameshift deletion	3	p.E145Gfs*10	c.426_448delCGTGCCGGAGAAGCGTTCCCA G	No
14	Heterozygosis	Nonsense	1	p.K84*	c.250A>T	No
16	Heterozygosis	Frameshift insertion	1	p.Y49Afs*4	c.144_145insGCAAAG	No
17	Heterozygosis	Cleavage site	4-5 ¹	/	c.598-1G>A	Yes
18	Heterozygosis	Nonsense	1	p.Y49*	c.147C>G	No
19	Heterozygosis	Frameshift deletion	2	p.K122Afs*40	c.363_364delGA	Yes
20	Homozygous	Cleavage site	3-4 ¹	/	c.464+1G>A	No

¹Mutation is located in the intron.

the *AXIN2* mutation in patient No. 7 were pathogenic based on the results of each software.

Relationship between gene mutation and clinicopathological parameters in patients with PJS

Relationship between mutations and family history: Of the 20 patients in this group, 11 had a family history and 9 had no clear family history. The sequencing results showed the following trend (Figure 1): PJS patients with a family history had a higher *LKB1/STK11* mutation rate than those without a family history (81.1% vs 77.8%, *PLKB1/STK11* = 1.000), and the incidence of *LKB1/STK11* truncation mutations was slightly higher than that in those without a family history (54.5% vs 44.4%, *Ptruncation mutation* = 1.000). In addition, the incidence of mutations in other genes was slightly lower than that in those without a family history (27.3% vs 66.7%, *Remaining genes* = 0.175). However, due to the small sample size in this group, no statistical difference was observed.

Relationship between mutations and age of dark spots: Of the 20 patients in this group, 11 had black spots aged ≤ 3 years and 9 had black spots aged > 3 years. The former was referred to as the early-onset group and the latter as the late-onset group. The sequencing results showed the following trend (Figure 1): Patients with PJS in the early-onset group had a higher *LKB1/STK11* mutation rate than those in the late-onset group (90.9% vs 66.7%, *PLKB1/STK11* = 0.285), and the incidence of *LKB1/STK11* truncation mutations was slightly higher than those in the late-onset group (54.5% vs 44.4%, *Pmutation* = 1.000). In addition, the incidence of mutations in other genes was slightly lower than those in the late-onset group (27.3% vs 66.7%, *Remaining genes* = 0.175). However, due to the small sample size in this group, no statistical difference was observed.

Relationship between mutation and clinical pathological parameters: The group was divided according to the presence or absence of *LKB1/STK11* mutations, presence or absence of *LKB1/STK11* truncation mutations, and other gene mutations. The Mann-Whitney *U* test was used to analyze the differences in polyp distribution, polyp load, and internal or surgical intervention. The results showed that the maximum diameter of colorectal polyps was greater in the presence of *LKB1/STK11* mutations (*U* = 32.000, *P* = 0.048), and the others were not statistically different (Table 7).

Follow-up

All patients of this study were followed-up to January 10, 2020. The final follow-up age was 25.9 ± 15.307 years, and the oldest patient was 47 years. The time span from

Table 3 Prediction of protein function and amino acid evolution conservation of *LKB1/STK11*

Case No.	Polyphen-2		Mutation taster		FATHMM		M-CAP		GERP++		phyloP	
	Score	Prediction	Score	Prediction	Score	Prediction	Score	Prediction	Score	Prediction	Score	Prediction
1	1	Probably damaging	1	Pathogenic	-2.5	Damaging	0.591	Damaging	5.6	Conserved	7.91	Conserved
2	/	/	1	Pathogenic	/	/	/	/	3.9	Conserved	8.998	Conserved
3	/	/	1	Pathogenic	/	/	/	/	/	/	/	/
4	/	/	1	Pathogenic	/	/	/	/	/	/	/	/
5	/	/	1	Pathogenic	/	/	/	/	/	/	/	/
6	0.993	Probably damaging	1	Pathogenic	-2.79	Damaging	0.704	Damaging	5.6	Conserved	7.799	Conserved
7	/	/	1	Pathogenic	/	/	/	/	/	/	/	/
8	/	/	/	/	/	/	/	/	/	/	/	/
10	/	/	/	/	/	/	/	/	/	/	/	/
13	/	/	1	Pathogenic	/	/	/	/	/	/	/	/
14	/	/	1	Pathogenic	/	/	/	/	3.9	Conserved	8.998	Conserved
16	/	/	1	Pathogenic	/	/	/	/	/	/	/	/
17	/	/	/	/	/	/	/	/	/	/	/	/
18	/	/	1	Pathogenic	/	/	/	/	3.9	Conserved	3.875	Conserved
19	/	/	1	Pathogenic	/	/	/	/	/	/	/	/
20	/	/	/	/	/	/	/	/	/	/	/	/

Case No. 8, 10, 17, and 20 are cleavage site mutations.

the patient's first admission was 8.9 ± 8.837 years. Five of these patients were re-admitted to our hospital for a total of 14 colonoscopy examinations and treatments.

DISCUSSION

In this patient group, 80.0% (16/20) of PJS cases were found to have *LKB1/STK11* mutations in hamartoma polyps, consistent with previous reports^[13-15]. In addition, 9 patients (45.0%) also had 18 types of mutations in other genes. The total incidence of mutations in this group of patients was 90.0% (18/20). Among them, *LKB1/STK11* gene: c.243delG, c.363_364delGA, c.722delC, c.144_145insGCAAG, c.454_455insC, c.464+1G>T, c.464+1G>A, c.598-1G>A, *MSH2*: C.792+1G>A, *MSH6*: c.3689C>G, c.4001+13C>CTTAC, *PMS1*: c.46C>T, and c.922G>A are newly discovered mutations, which suggest that the genetic mutations in PJS hamartoma polyp tissue are complex and diverse. In addition, we found that the cases with mutations in the exon 5 of *LKB1/STK11* gene were all in the early-onset group and the cases with splice site mutations in the exon 3 were all in the late-onset group. Those with negative *LKB1/STK11* mutations but carrying other gene mutations were all in the late-onset group. This suggests that different clinical phenotypes of PJS may have a different molecular genetics basis. This is worth further study.

The clinical phenotypic heterogeneity of PJS is obvious. With the continuous improvement in gene detection technology, the relationship between genotype and clinical phenotype has become a focus. However, PJS has scattered populations and relatively few cases as it is also a very rare disease in the clinic. A lot of research has been carried out at home and abroad, but no consensus has been reached on the relationship between genotypes and clinical phenotypes. Although this study did not detect a statistically significant mutation frequency in patients with or without a family history due to the small sample size, we found that the colorectal polyps with *LKB1/STK11* mutations were larger ($U = 32.000$, $P = 0.048$). There was no statistically significant relationship between whether *LKB1/STK11* gene was mutated and whether it was a truncation mutation and the patient's polyp distribution, polyp load, polyp size, and medical or surgical intervention. Some studies have demonstrated that MLPA assay technology can improve the detection rate in *LKB1/STK11* gene mutation screening in PJS patients^[16]. If the MLPA assay is performed in patients with negative mutations, there may be new findings. However, we also found that two patients with *LKB1/STK11* gene exon 5 anterior and posterior splicing site mutations had early-onset of pigment spots, and two patients with cleavage site mutations in exon 3 had late-onset of pigment spots, and patients without *LKB1/STK11* gene mutations but

Table 4 Mutation of other 13 genes except *LKB1/STK11* gene

Case No.	Gene	MMR	Type of mutation	Amino acid change	Base change	New mutation
3	<i>MUTYH</i>	No	Missense	p.Ala373Val	c.1118C>T	No
	<i>MLH1</i>	Yes	Missense	p.Val384Asp	c.1151T>A	No
	<i>PMS2</i>	Yes	Missense	p.Thr511Met	c.1532C>T	No
4	<i>MSH6</i>	Yes	Missense	p.Ala1230Gly	c.3689C>G	Yes
	<i>MLH1</i>	Yes	Missense	p.Val384Asp	c.1151T>A	No
	<i>PMS2</i>	Yes	Missense	p.Thr511Met	c.1532C>T	No
7	<i>MLH3</i>	Yes	Non-synonymous SNV	p.Asp1081His	c.3241G>C	No
	<i>AXIN2</i>	No	Non-synonymous SNV	p.Ser738Phe	c.2213C>T	No
9	<i>MSH6</i>	Yes	Missense	p.Glu1163Val	c.3488A>T	No
	<i>APC</i>	No	Missense	p.Met2221Thr	c.6662T>C	No
10	<i>MSH2</i>	Yes	Missense	p.Ile169Val	c.505A>G	No
	<i>MSH6</i>	Yes	Intron insertion	/	c.4001+13C>CTTAC	Yes
	<i>APC</i>	No	Missense	p.Ala2778Ser	c.8332G>T	No
14	<i>MSH2</i>	Yes	Missense	p.Val89Ala	c.266T>C	No
	<i>MSH2</i>	Yes	Cleavage site	/	c.792+1G>A	Yes
	<i>PMS1</i>	Yes	Nonsense	p.Gln16Ter	c.46C>T	Yes
	<i>PMS1</i>	Yes	Missense	p.Val308Ile	c.922G>A	Yes
15	<i>PTEN</i>	No	Missense in 5' untranslated region (UTR)	p.Gln171Glu	c.511C>G	No
19	<i>MSH2</i>	Yes	Missense	p.Leu390Phe	c.1168C>T	No
20	<i>MLH1</i>	Yes	Missense	p.Arg217Cys	c.649C>T	No

with other gene mutations all had late-onset of pigment spots. Limited by the sample size in this study, there was no statistical difference between the two groups, and we may be able to uncover the molecular genetic mechanism of clinical subtypes if the sample size is increased in further studies.

In addition, the mutation rate of *LKB1/STK11* gene in PJS patients has not reached 100% using various sequencing techniques, which may be related to the limitations of current technology, but it is more likely to suggest that PJS is a heterogeneous genetic disease, or that there are signaling pathways related to its development and progression. Moreover, we also found that there were other gene mutations in the PJS hamartoma polyp tissue, in which the *DNA mismatch repair (MMR)* gene is particularly prominent (accounting for 88.9% of all other gene mutations). According to a variety of software predictions, 81.8% (9/11) of them may be pathogenic and conservative in amino acid evolution. These may be the inherent genetic mechanism of the clinical phenotypic heterogeneity of PJS. The MMR system mainly includes proteins such as hMLH1, hMSH2, hMSH3, hMSH6, hPMS1, and hPMS2, which maintain gene stability mainly by repairing mismatched bases and insertion/deletion loops in DNA synthesis^[17-19]. Among them, MSH2 and MSH6, MSH2 and MSH3 constitute MutS α and MutS β , respectively. The former can recognize single base mismatch and insertion/deletion loops, and the latter can recognize 2-8 base insertion/deletion loops. However, MutL α and MutL β are composed of MLHL with PMS2 and PMSL, and their functions are to localize the mismatch site, cooperate with Exo I, proliferating cell nuclear antigen, and DNA polymerase to remove base mismatches and resynthesize the correct DNA^[20]. Functional alterations in MMR may cause microsatellite instability, which can be found in sporadic and hereditary tumors in various tissues^[21-23], and have clear guiding significance for prognosis and drug efficacy prediction in colorectal cancer patients. In particular, only *MSH6* mutation was detected in the PJS hamartoma polyps without *LKB1/STK11* mutation. This also indicates that there may be other mechanisms besides *LKB1/STK11* involved in the occurrence, development and malignant transformation of PJS hamartoma polyps. Therefore, we consider that destruction of the MMR system may play an important role in the development course of some PJS patients, and with the continuous accumulation of DNA replication errors, it leads to an increased risk of malignant transformation in various tissues and organs. This is worthy of further study.

It was reported that the risk of intussusception in PJS patients was 50% at age 20 years, the incidence of intestinal intussusception was 95%, and 80% of intussusceptions manifested as acute abdomen and 92.5% of cases were treated with surgery^[9]. All patients in the present group did not experience intestinal obstruction,

Table 5 Prediction of protein function changes caused by *MSH6* and other gene mutations

Case No.	Gene	Polyphen-2_HDIV		Mutation Taster		FATHMM		M-CAP	
		Score	prediction	Score	Prediction	Score	Prediction	Score	Prediction
3	<i>MUTYH</i>	0.069	Benign	1	Pathogenic	-2.41	Damaging	0.084	Damaging
3	<i>MLH1</i>	1	Probably_damaging	1	Pathogenic	-2.66	Damaging	/	/
3	<i>PMS2</i>	0.03	Benign	1	Polymorphism	1.06	Tolerable	/	/
4	<i>MSH6</i>	1	Probably_damaging	1	Pathogenic	-2.52	Damaging	0.292	Damaging
4	<i>MLH1</i>	1	Probably_damaging	1	Pathogenic	-2.66	Damaging	/	/
4	<i>PMS2</i>	0.239	Benign	1	Polymorphism	1.06	Tolerable	/	/
7	<i>MLH3</i>	1	Probably_damaging	1	Pathogenic	-2.37	Damaging	0.137	Damaging
7	<i>AXIN2</i>	0.121	Benign	0.997	Polymorphism	-0.25	Tolerable	/	/
9	<i>MSH6</i>	0.67	Probably_damaging	1	Pathogenic	-2.12	Damaging	/	/
9	<i>APC</i>	0.156	Benign	0.737	Pathogenic	-2.47	Damaging	0.046	Damaging
10	<i>MSH2</i>	0	Benign	1	Polymorphism	-2.29	Damaging	0.028	Damaging
10	<i>MSH6</i>	/	/	/	/	/	/	/	/
10	<i>APC</i>	1	Probably_damaging	1	Pathogenic	-1.53	Damaging	0.033	Damaging
14	<i>MSH2</i>	0.042	Benign	1	Pathogenic	-2.47	Damaging	0.075	Damaging
14	<i>MSH2</i>	/	/	/	/	/	/	/	/
14	<i>PMS1</i>	/	/	1	Pathogenic	/	/	/	/
14	<i>PMS1</i>	0.329	Benign	0.996	Pathogenic	-1.34	Tolerable	0.03	Damaging
15	<i>PTEN</i>	0.956	Probably_damaging	0.999	Pathogenic	/	/	/	/
19	<i>MSH2</i>	0.148	Benign	1	Pathogenic	-3.07	Damaging	/	/
20	<i>MLH1</i>	1	Probably_damaging	1	Pathogenic	-1.91	Damaging	0.247	Damaging

intussusception or other gastrointestinal emergencies and malignant changes of polyps during the follow-up period, and did not undergo surgical treatment. We believe that high-frequency enteroscopy and microscopic treatment effectively alleviate the progress of the disease and prolong the patient's survival.

Table 6 Prediction of amino acid evolutionary conservation due to mutations in *MSH6* and other genes

Case No.	Gene	GERP++		phyloP	
		Score	Prediction	Score	Prediction
3	<i>MUTYH</i>	5.67	Conserved	6.955	Conserved
3	<i>MLH1</i>	5.67	Conserved	7.336	Conserved
3	<i>PMS2</i>	-3.23	Nonconserved	-0.25	Nonconserved
4	<i>MSH6</i>	5.5	Conserved	7.481	Conserved
4	<i>MLH1</i>	5.67	Conserved	7.336	Conserved
4	<i>PMS2</i>	-3.23	Nonconserved	-0.25	Nonconserved
7	<i>MLH3</i>	4.6	Conserved	5.502	Conserved
7	<i>AXIN2</i>	2.07	Conserved	2.225	Conserved
9	<i>MSH6</i>	5.23	Conserved	8.923	Conserved
9	<i>APC</i>	6.02	Conserved	3.925	Conserved
10	<i>MSH2</i>	-1.25	Nonconserved	1.857	Nonconserved
10	<i>MSH6</i>	/	/	/	/
10	<i>APC</i>	5.92	Conserved	8.947	Conserved
14	<i>MSH2</i>	3.94	Conserved	3.331	Conserved
14	<i>MSH2</i>	/	/	/	/
14	<i>PMS1</i>	4.99	Conserved	7.805	Conserved
14	<i>PMS1</i>	2.11	Conserved	4.333	Conserved
15	<i>PTEN</i>	/	/	/	/
19	<i>MSH2</i>	4.62	Conserved	1.611	Nonconserved
20	<i>MLH1</i>	5.76	Conserved	2.993	Conserved

Table 7 Relationship between gene mutation and clinical pathological parameters

Mutation	Result	Load of gastric polyps	Maximum diameter of gastric polyps (mm)	Load of duodenal and small intestinal polyps	Maximum diameter of duodenal and small intestinal polyps (mm)	Load of Colorectal polyps	Maximum diameter of colorectal polyps (mm)	Number of hospitalization times	Number of operation times	Number of intervention times
<i>LKB1/STK11</i> mutations	U value	28.000	30.000	35.500	26.000	20.500	32.000	36.000	49.000	28.500
	P value	0.885	1.000	0.442	0.878	0.734	0.048	0.750	0.122	0.750
<i>LKB1/STK11</i> truncating mutation	U value	62.500	69.000	49.500	47.000	23.500	35.500	56.000	40.500	35.500
	P value	0.156	0.053	0.436	0.605	0.613	0.397	0.684	0.481	0.280
Other gene mutations	U value	47.500	42.500	39.000	36.000	22.000	19.500	38.000	46.000	41.500
	P value	0.842	0.842	0.965	0.762	0.607	0.388	0.412	0.824	0.552

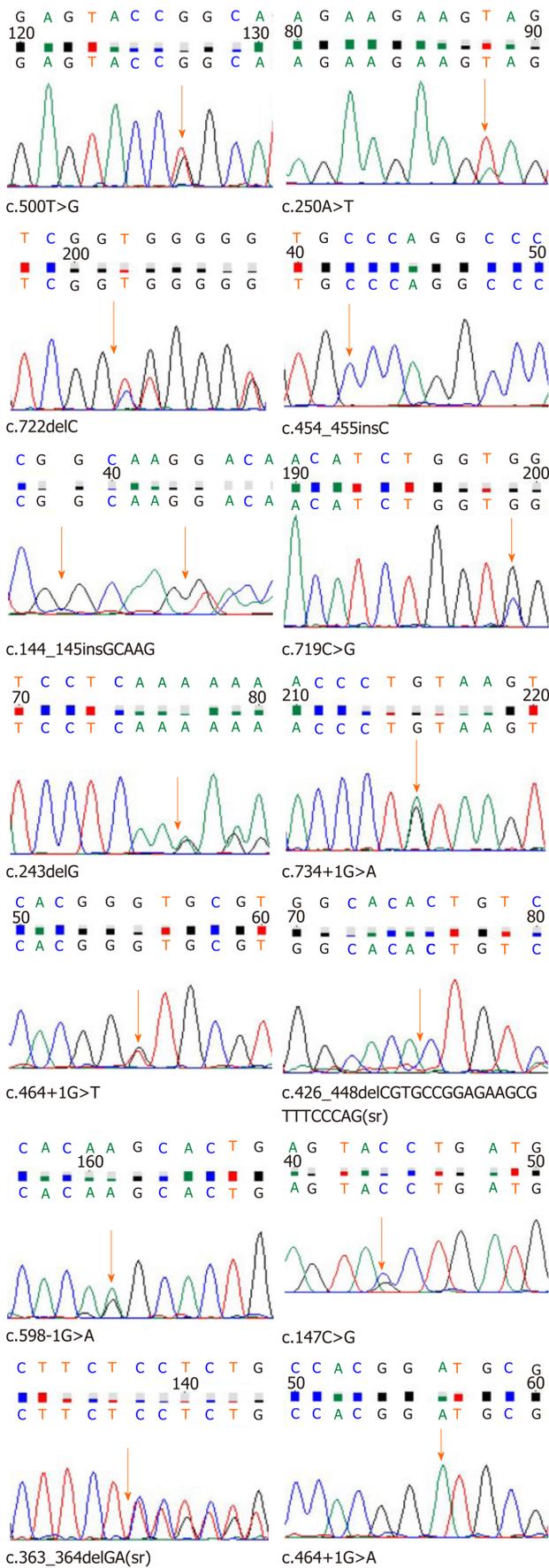


Figure 1 Peak map of *LKB1/STK11* mutation sequencing. The arrow points to the mutation position. "sr" represents reverse sequencing and the remainder is forward sequencing.

ARTICLE HIGHLIGHTS

Research background

Peutz-Jeghers syndrome (PJS) is a rare autosomal dominant genetic disease, which belongs to the category of hereditary colorectal cancer. It is currently believed that the occurrence of PJS is closely related to mutations in the *LKB1/STK11* gene, and that different types of mutations have different effects on clinical phenotype. The genetic heterogeneity of PJS is obvious, and no other pathogenic genes have been found except the *STK11* gene, and the relationship between genotype and phenotype is not clear.

Research motivation

This study aimed to investigate the mutation status of hereditary colorectal tumor-associated genes in hamartoma polyp tissue of PJS and discuss its relationship with the clinicopathological data of PJS.

Research objectives

To investigate mutations in genetically-related genes, try to explain the genetic heterogeneity of the disease, and investigate whether the disease has a relatively clear genotype-phenotype relationship.

Research methods

Twenty patients with PJS were randomly selected for this study who were treated in the Air Force Medical Center and their clinicopathological data were collected, including family history, polyp distribution, polyp load, and internal or surgical intervention. Next-generation sequencing technology was used to study the mutation status of the genetically-related genes in PJS hamartoma polyp tissues, and examine the relationship between the mutation status of these genes and the clinical pathological data of PJS.

Research results

LKB1/STK11 gene mutations were detected in 16 of 20 cases, with 14 types of mutations, among which 8 new mutations were detected. 18 types of other gene mutations were detected in 9 of these 20 cases, all of which were heterozygous mutations. There was no statistical difference between mutations and family history, and between mutations and blackspot age. The maximum diameter of colorectal polyps was greater in the presence of *LKB1/STK11* mutations.

Research conclusions

We found a series of gene mutation types in hamartoma polyp tissues of PJS patients, and destruction of the MMR system may play an important role in the development course of some PJS patients. The colorectal hamartoma polyps with *LKB1/STK11* mutations were larger than those with other gene mutations.

Research perspectives

Improvements in gene sequencing technology and the identification of new mutation sites of *STK11* and other possible pathogenic genes are necessary to describe the pathogenesis of PJS at the genetic level. In addition, an investigation into whether the disease has a relatively clear genotype-phenotype relationship is a hot spot for future research.

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