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ORIGINAL ARTICLE

Basic Study

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

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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

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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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.

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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