Cancer Genetics 230 (2019) 47–57

Cancer Genetics

ORIGINAL ARTICLE

STK11 gene analysis reveals a significant number of splice mutations in Chinese PJS patients

Yu-Liang Jiang[a,b,1], Zi-Ye Zhao[c,1], Bai-Rong Li[b], Hao Wang[c], En-Da Yu[c,*], Shou-Bin Ning[b,*]

[a] *Department of Gastroenterology, Shijitan Hospital, 10 Tieyi Rd., Beijing 100038, China;* [b] *Department of Gastroenterology, Airforce Specialty Medical Center of PLA, 30 Fucheng Rd., Beijing 100142, China;* [c] *Department of Colorectal Surgery, Changhai Hospital, 168 Changhai Rd., Shanghai 200433, China*

Abstract

Background: The combination of direct sequencing and multiple ligation-dependent probe amplification (MLPA) has resulted in an 80% detection rate of serine/threonine kinase 11 (*STK11*) gene mutations in Peutz-Jeghers syndrome (PJS); however, this rate varies in different ethnicities.

Aims: To test the efficacy of the combination in Chinese patients with PJS.

Methods: PJS probands visiting our center during one year were enrolled. Sanger sequencing and MLPA were used to detect *STK11* mutations. Associations between the occurrence of severe complications and risk factors were analyzed statistically.

Results: We identified 47 PJS probands. Among them, 34 received an *STK11* mutation test, revealing 23 point mutations and 2 exonic deletions. Nine of the mutations were splicing errors, reflecting a significantly higher proportion ($p < 0.05$). Laparotomy history existed for 33 of the probands, and seven families had a history of cancer. Statistical analysis revealed no associations between the occurrence of severe complications or cancers and risk factors.

Conclusion: The strategy achieved a high detection rate in Chinese people, validating its effectiveness. This cohort comprised a significantly higher proportion of splicing errors, reflecting the unique genetic characteristics Chinese people. No specific genotype-phenotype relationship was noted, while the wide usage of enteroscopy would benefit PJS surveillance.

Keywords Peutz-Jeghers syndrome, STK11, Cancer, Surveillance, Multiple ligation-dependent probe amplification, MLPA.

© 2018 Elsevier Inc. All rights reserved.

Introduction

Peutz-Jeghers syndrome (PJS; OMIM #175200) is an autosomal dominant inherited disorder, characterized by mucocutaneous pigmentation (MP), hamartomatous polyps of the gastrointestinal (GI) tract (PJPs), and an increased risk of developing of various neoplasms [1,2]. PJS is also a type of colorectal cancer (CRC syndrome. Family history (FH) is positive in certain cases, whereas others arise *de-novo*. The incidence of PJS is estimated to be low, at 0.5 to 2 in 100,000 [3]; however, the incidence in a specific country or area is unknown.

As a Mendelian disorder, PJS's genetic cause has been identified as germline mutations in the serine–threonine kinase 11 (*STK11*) gene [4,5]. This gene is located on chromosome 19p13.3 and comprises nine exons that encode a 433 amino-acid protein. As a tumor suppressor, STK11 works together with several partners, like STE20-related kinase adaptor alpha (STRADA) and scaffold protein 25 (MO25) [6], and takes part in many biological processes and signaling pathways, such as cell cycle arrest [7], P53-mediated

Abbreviations: DBE, double-balloon enteroscopy; DHPLC, denaturing high performance liquid chromatography; EGD, esophagogastroduodenoscopy; GI, gastrointestinal; MLPA, multiplex ligation-dependent probe amplification; MP, melanin pigmentation; NGS, next generation sequencing; PCR, polymerase chain reaction; PJP, Peutz-Jeghers syndrome polyp; PJS, Peutz-Jeghers syndrome; PRP, prolapsed rectal polyp; SSCP, single strand conformation polymorphism; *STK11*, Serine/threonine kinase 11.

Received July 13, 2018; received in revised form October 2, 2018; accepted November 27, 2018

* Corresponding authors.

E-mail addresses: endayu@yeah.net, ningshoubin@126.com

[1] These authors contributed equally to this work.

2210-7762/$ - see front matter © 2018 Elsevier Inc. All rights reserved.
https://doi.org/10.1016/j.cancergen.2018.11.008

apoptosis [8], Wnt signaling [9,10], transforming growth factor beta (TGF-β) signaling [11], Ras induced cell transformation [12], and cell polarity [13–16]. Its extensive participation in biological processes means that functional mutations of *STK11* probably cause physiological malfunction in PJS and neoplasms in multiple sites [17].

The diagnosis of PJS consists of two aspects, clinical and genetic. The clinical criteria are taken from the comprehensive system in the Mallorca consensus statement (2007), which allows for any two of the three features (PJP, MP, and FH) or the isolated presence of two or more PJPs to diagnose PJS [3]. The genetic diagnosis is based on the identification of an *STK11* germline mutation, although a single study has found an *MYH11* (encoding myosin heavy chain 11) germline mutation in a PJS family [18]. The detection methods include Sanger sequencing, next generation sequencing (NGS), denaturing high performance liquid chromatography (DHPLC), single strand conformation polymorphism (SSCP), and multiple ligation-dependent probe amplification (MLPA). Until now, Sanger sequencing plus MLPA, or NGS plus MLPA, have been the best combined detection strategies, with detection rates of 65.8–87.5% [19–26]. The effectiveness of the combination may vary when applied in different ethnicities. Chinese Han people are one of the most populous races, accounting for more than 20% of the human population; however, data from China are very limited. Previous reports presented PJS detection rates of 67.3% and 81.8%, respectively, using these combinations, which is a marked difference.

In the present study, we applied the combination of direct sequencing and MLPA in a cohort of patients with PJS hospitalized over the course of one year to explore the effectiveness of this strategy in Chinese Han people. We focused on the risk factors that may be associated with the occurrence of polyp-related complications and tumorigenesis.

Materials and methods

Subjects

A total of 54 patients were diagnosed clinically with PJS in the Airforce General Hospital of PLA (Beijing) and Changhai Hospital (Shanghai) between Sep 2016 and Aug 2017. The clinical diagnostic criteria of PJS, as recommended by WHO were adopted: (1) Three or more histologically PJPs in the gastrointestinal tract; (2) any number of PJPs detected with a positive FH of PJS; (3) characteristic MP with a positive FH of PJS; and (4) any number of PJPs together with characteristic MP [27]. The presence of any one of the above clinical findings would suggest a clinical diagnosis of PJS. For inpatients, surgery or GI endoscopic intervention, which included esophagogastroduodenoscopy (EGD), colonoscopy, double-balloon enteroscopy (DBE), or capsule endoscopy, were performed. Outpatients received EGD, colonoscopy, or only a genetic test. Fifty unrelated ethnicity-matched individuals who came to Airforce General Hospital of PLA for gastric polyps' treatment during the same time period were also included in the study as the control group.

Genetic tests were performed based on the probands' genograms. Blood samples of the probands and all available family members were collected after obtaining informed consent. This study was approved by the Medical Ethics Committee, Airforce General Hospital of PLA, and the Institutional Review Board of Changhai Hospital.

Genomic DNA isolation

Peripheral blood samples of at least 5 ml were collected in ethylenediaminetetraacetic acid (EDTA) anti-coagulated tubes. Genomic DNA from peripheral blood leucocytes was extracted routinely using an animal genomic DNA kit (TSP201, TsingKe Biotech, Beijing, China) according to the manufacturer's instructions. The extracted DNA was stored at $-20\,^{\circ}$C before subsequent analyses.

Direct sequencing

All nine coding exons and their flanking sequences (20 bp) of the *STK11* gene were amplified using the primers listed in Table S1. Polymerase chain reactions (PCR) of *STK11* exons were performed in a 50-µl reaction system that contained 10 µM of each primer, 100 ng of genomic DNA, and 25 µl of $2\times$ TsingKe master mix polymerase (TSE004, TsingKe Biotech, Beijing, China). The PCR reactions were performed according to the manufacturer's instructions. The PCR products were gel- and column-purified using a purification kit (GE0101, TsingKe Biotech, Beijing, China). The purified PCR fragments were then sequenced using the BigDye Terminator method on an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA) by Majorbio Co. Ltd. (Shanghai, China). The results were used to perform sequence alignment according to *STK11* gene sequence (NP_000446.1 and NM_000455.4 in GRCh38.p7). The experimental details have been reported previously [28,29].

All available family members underwent an *STK11* germline mutation test to confirm cosegregation of the mutation with the disease. To rule out polymorphisms and to confirm the pathogenic effect of the variations, 100 chromosomes of 50 individuals from the control group were also screened for the presence of the mutations.

Multiplex ligation-dependent probe amplification (MLPA) assay

The SALSA MLPA reagent kit (EK1-FAM, MRC-Holland, Amsterdam, the Netherlands) and SALSA MLPA probemix *STK11* (P101-B4) were prepared. The sequences of probes used are shown in Table S2. The SALSA MLPA reagent kit containing SALSA MLPA buffer, Ligase-65, Ligase buffer A and B, SALSA PCR primer mix, and SALSA polymerase, was prepared. Extracted genomic DNA (100 ng) was denatured by incubating at 98 °C in a thermocycler, and then the MLPA probes were added to the genomic DNA samples and hybridized at 60 °C. The subsequent ligation reaction was performed at 54 °C for 15 min after the addition of the ligase and ligase buffer. The ligase was inactivated by incubating the reaction tube at 98 °C. PCR amplification of the ligation product was performed following the addition of universal PCR primers, dNTPs, and polymerase. The amplified products were then analyzed using the ABI 3730XL DNA Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) by Sangon Co.

Ltd. (Shanghai, China). The MLPA assay results were analyzed using Coffalyser.Net (MRC-Holland, Amsterdam, The Netherlands). A peak ratio less than 0.7 was interpreted as a "deletion", while a peak ratio greater than 1.3 was interpreted as a "duplication".

Statistical analysis

Occurrence of severe polyp-related complications (presented at laparotomy) and tumorigenesis are the two main concerns in PJS prognosis; therefore, we explored the association between them and certain risk factors (gender, family history of PJS, and *STK11* mutation information) by statistical analysis. Statistical analyses of the data were performed using SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, USA). The results obtained were expressed in terms of median and range or proportion. χ^2 test or Fisher's exact test were used to test the significance, which was set at $p < 0.05$.

Results

Clinical features

The study process was performed according to the flowchart displayed in Fig. S1. During the whole year investigated (Sep 2016–Aug 2017), 54 patients with clinically diagnosed PJS visited our hospitals and were enrolled in this investigation. The median age of this cohort for diagnosis and first intervention (polypectomy or laparotomy) were 18 and 17 years, respectively, and the proportion of men was 70.4%. Among them, 24 had family history of PJS and 30 did not. Thirty-four patients experienced severe complications, such as intussusception, bowel obstruction, and tumorigenesis. The clinical characteristics are presented in Table 1.

After sorting according to family history, 47 patients were determined as probands of the PJS pedigrees, upon which the subsequent analyses were based. As shown in Table 1, the 47 probands from unrelated families comprised 17 familial, 29 sporadic patients, and 1 from an adoptive family, and their birth places comprised various locations in China (Table S3). The 17 familial cases (PJS02, 03, 05–07, 13, 14, 30, 33, 35–39, 41, 44, and 45) showed an autosomal dominant pattern of inheritance (Fig. 1). MP on the lips, buccal mucosa, or digits (Fig. 2(A)–(C)) and classical PJPs in the GI tract (Fig. 2(G)) were detected in all probands. All probands in these families underwent polypectomy, using open surgery or endoscopy, at various ages, and 32 of them underwent laparotomy because of intussusception, intestinal obstruction, or other complications (Fig. 2(D) and (E)). Fifteen probands underwent DBE. Four probands (PJS07, 12, 29, and 40) in this study had developed PJS-related tumors (GI tract cancers and genital tumors) before the age of 35 years (Fig. 2(F)), and familial cancer history existed in another three families (PJS14, 41 and 45). Detailed information is shown in Table 2.

Direct sequencing

Mutation detection by direct sequencing was performed in 34 probands, and mutations in the *STK11* gene were observed in 23 of them (67.6%). Missense, nonsense, indel, and splicing mutations were observed in five, five, four, and nine probands, respectively (Table 2 and Fig. 3). In particular, one of the indels (c.907_915delATCCGGCAG) was the deletion of three codons, which resulted in the deletion of three amino acids instead of a frame shift (p.303_305delIRQ). Except for one mutation (c.109C > T) in the region encoding the N-terminal domain, all the other mutations were located within the kinase domain coding region of the *STK11* gene. Most of the mutations have been previously reported [30,31] except for c.243delG, c.667G > T, c.464 + 1G > A (IVS3), and c.598–1G > A (IVS4). Details about mutation 667G > T are presented in Fig. S2. All three frame shift mutations led to partial loss of the kinase domain and completed loss of the C-terminus. PJS10, 12, 28, 31, and 40 possessed nonsense mutations of *STK11* that resulted in the production of truncated proteins (p.Y60*, p.E223*, p.Q37*, p.W239*, and p.Q220*). Among the 23 patents with mutations, nine were familial, 13 were sporadic, and one of them was adopted with an unclear family history.

MLPA assay

Nine probands received direct sequencing without pathogenic mutations being detected; therefore, they were subjected to an MLPA assay. Among them, a deletion in exon 1 of *STK11* was observed in two probands with PJS (Fig. 3).

Therefore, by applying both direct sequencing and the MLPA assay, the total mutation detection rate for *STK11* gene in this Chinese cohort with PJS was 73.5% (25 of 34 probands).

Polyp-related complications, tumorigenesis, and risk factors

Polyp-related complications include abdominal pain and distention, diarrhea, hematochezia, prolapsed rectal polyp (PRP), intussusception, and bowel obstruction. Among them, intussusception and bowel obstruction are the most severe, which often require treatment by laparotomy. There were 32 probands who received laparotomy, and none of the risk factors, including gender, family history of PJS, *STK11* mutation detected, or *STK11* splicing error was associated with requirement for laparotomy ($p > 0.05$) (Table S4). Tumorigenesis occurred in seven of the PJS families, and none of the risk factors mentioned above was associated tumorigenesis ($p > 0.05$) (Table S5).

Discussion

PJS has various manifestations related to PJPs, such as abdominal pain, distention, hematochezia, chronic anemia, PRP, bowel obstruction, and clinical intussusception [32–34]. An increased risk of cancer at multiple sites is also a considerable threat to patients with PJS, and the sites range widely, including the GI tract, breast, ovary, testis, and lung [35]. The two main purposes of PJS management/surveillance are to detect sizeable GI polyps and to detect cancer at the early

Table 1 Characteristics of the cohort with PJS.

Characteristics		Number (percentage)	
For all PJS patients			
Cases diagnosed in the study period		54	
Age*	*D*	18 (1–55) years	
Median (range)	*F*	17 (1–42) years	
	P	24 (4–56) years	
Gender			
Male		38	70.4%
Female		16	29.6%
Family history**			
Familial		24	45.3%
Sporadic		29	54.7%
Severe GI complications***			
Yes		36	69.2%
No		16	30.8%
For PJS probands			
Probands in duration		47	
Gender			
Male		33	70.2%
Female		14	29.8%
Family history**			
Familial		17	37.0%
Sporadic		29	63.0%
Severe GI complications***			
Yes		33	73.3%
No		12	26.7%
DNA mutation analysis		34/47	72.3%
STK11 mutation carrier		25/34	73.5%

* *D*, diagnosis; *F*, first polypectomy or laparotomy; *P*, present. Present age was unknown for one patient and age at diagnosis and polypectomy were unknown for two patients.

** One patient was excluded from the analyses because he was from an adoptive family.

*** Two patients were excluded from the analyses because of lack of information. PJS, Peutz-Jeghers syndrome; GI, gastrointestinal.

stage [3]. In the present study, we noticed that a history of laparotomy and PJS-related cancer existed in 32/45 (71.1%) and 7/45 (15.6%) of the probands, respectively. The high prevalence validates the necessity of close follow up in patients with PJS. PJS-related cancers can occur in a patient's early twenties or thirties, in addition to other PJS-related complications that occur in young children; therefore, the use of baseline and follow-up endoscopy must be emphasized, and may be performed earlier and more strictly. Belsha et al. [36] suggested that DBE-facilitated polypectomy is an effective therapeutic option in pediatric patients with PJS. Among 16 patients, 14 underwent DBE-facilitated polypectomy (11 by DBE alone and three by Lap-DBE facilitated polypectomy). Except for one pelvic abscess post-procedure, which was related to an infected laparoscopy-port, all other patients recovered well and lived uneventfully in the 26 month follow up. Compared with these results, we believe that the high prevalence of laparotomy observed in the present study reflects the lack of DBE usage in China. Many open surgeries could be avoided if DBE was used earlier. Statistical analysis did not reveal significant associations between the occurrence of severe complications or cancers and risk factors like gender, FH, *STK11* mutation detection, or *STK11* splicing errors ($p > 0.05$) (Table S4 and S5).

Among *STK11* mutations recorded in the Human Genome Mutation Database (HGMD), more than 300 point mutations and small range mutations are recorded, and 89 gross deletions or insertions [37]. As a cause of mutations, genomic rearrangements have been detected by MLPA and reported in other hereditary CRC syndromes, such as Lynch syndrome [38] and familial adenomatous polyposis (FAP) [39]. Discovery of the pathogenic mutation is the cornerstone of genetic counseling; however, it is not always possible because of technical limitations. Before MLPA, the detection rate of PJS was around 60% or lower [40–42]. With the help of MLPA, the detection rate has increased by 10–20% as shown in Table 3. In the present study, Sanger sequencing detected 23 mutations, and MLPA detected two additional mutations.

As the major pathogenic gene in PJS, *STK11* has been studied to identify associations between its mutations and elevated cancer risk [17,43–48]. Mehenni et al. [45] reported 149 patients with PJS s from 41 families, and suggested that mutations within exon 6 possessed a higher cancer risk. Salloch et al. [49] reported of 31 patients from 22 families, and suggested that truncating mutations were associated with more polyps, surgical interventions, and cancers. Wang et al. [26] reported 116 patients from 52 families, and stated that mutations in exon 7 correlated with a 90% (9/10) incidence of GI polyp

Table 2 Demography, initial evaluation, and clinical course of 47 PJS probands.

ID and Sex		Age*				Reason for visit	Laparotomy	Intervention	Tumorigenesis		Mutation		
		P	MP	*D*	*F*				Age	Tumor	Exon	Nucleotide	Amino acid change/effect
01	M	31	0	1	1	PRP	Yes	TE, Lap, Ent			1	del(exon1)	Kinase domain deletion
02	M	4	1	4	4	PRP	No	Ent			Not tested		
03	M	11	1	5	5	FH	Yes	Col, Lap, Ent			6	790–793delTTTG	F264RfsX22
04	M	28	0	5	5	Sto	Yes	Lap, EGD, Col, Ent				No mutation detected	
05	M	6	2	6	6	PRP	No	EGD, Col			4	597 + 1G > T(IVS4)	Splicing error
06	M	19	1	6	6	Int	Yes	Lap, Ent			7	863–2A > G(IVS6)	Splicing error
07	M	32	5	6	6	Int	Yes	Lap, Ent	32	CRC	5	598–1G > A(IVS4)***	Splicing error
08	M	9	0	7	7	Hemato	No	Col, Ent			1	243delG***	K81KfsX15
09	M	7	3	7	6	NR	Yes	Lap			Not tested		
10	F	17	2	9	9	BO	Yes	Lap			1	180C > G	Y60X
11	M	14	3	9	12	MP	No	Col, Ent			Not tested		
12	F	27	5	10	10	Hemato	Yes	Lap	26	SC	5	667G > T***	E223X
13	M	13	2	10	10	Int	Yes	Lap				No mutation detected	
14	F	24	0	11	11	FH	Yes	Lap	30**	CRC	7	863–2A > G(IVS6)	Splicing error
15	M	22	7	11	11	NR	Yes	Lap			Not tested		
16	M	18	4	12	12	MP	Yes	EGD, Col, Lap			6	735–1G > A(IVS5)	Splicing error
17	F	23	3	12	12	Int	Yes	Lap				No mutation detected	
18	F	14	4	13	13	Int	Yes	Lap			Not tested		
19	M	27	5	14	14	Hemate	No	EGD, Ent			4	580G > A	D194N
20	M	25	1	17	17	BO	Yes	Col, Lap			5	725G > A	G242E
21	M	23	1	18	18	Dia	No	Ent			4	580G > A	D194N
22	F	20	0	18	18	Int	Yes	Lap			7	910C > T	R304W
23	M	20	2	18	18	NR	Yes	Lap			Not tested		
24	*M*	20	NR	19	19	NR	No	EGD, Col				No mutation detected	
25	M	25	4	19	19	Int	Yes	Lap				No mutation detected	
26	F	27	4	19	19	Int	Yes	Lap			Not tested		

(*continued on next page*)

Table 2 (*continued*)

ID and Sex		Age*				Reason for visit	Laparotomy	Intervention	Tumorigenesis		Mutation		
		P	MP	*D*	*F*				Age	Tumor	Exon	Nucleotide	Amino acid change/effect
27	M	19	0	19	10	Int	Yes	Lap			Not tested		
28	M	34	4	20	20	NR	Yes	Lap			1	109C > T	Q37X
29	M	29	5	20	20	MP	Yes	Lap	29	SBC	1	157delG	D53TfsX11
30	M	33	10	21	21	Int	Yes	Lap			1	290 + 1G > A(IVS1)	Splicing error
31	F	23	NR	22	22	Hemato	No	EGD, Ent			5	716G > A	W239X
32	M	39	0	22	15	NR	Yes	Lap			7	907_915delATCCGGCAG	303–305delIRQ
33	M	23	0	23	23	NR	No	Ent			3	464 ± 1G ≥ A(IVS3)***	Splicing error
34	F	28	18	23	23	BO	Yes	EGD, Lap				No mutation detected	
35	M	26	0	25	25	MP	No	EGD, Col				No mutation detected	
36	M	36	0	25	17	Int	Yes	Lap, Ent			4	527A > G	D176G
37	F	33	2	26	20	Vom	Yes	Lap			Not tested		
38	F	36	3	27	10	Int	Yes	Lap, Ent			Not tested		
39	M	36	0	27	27	Sto	Yes	Col, Lap, Ent			2	291–2A > C(IVS1)	Splicing error
40	F	32	3	31	31	NR	Yes	Lap	31	OMC	5	658C > T	Q220X
41	M	32	4	32	7	Int	Yes	Lap	NR**	RC		No mutation detected	
42	M	41	10	34	18	NR	Yes	Lap			1	290 + 1G > T(IVS1)	Splicing error
43	F	36	5	34	34	Int	Yes	Lap			1	del(exon1)	Kinase domain deletion
44	M	41	1	35	35	NR	No	EGD, Col			Not tested		
45	M	42	1	38	38	NR	No	Col	NR**	SBC	Not tested		
46	F	NR	NR	NR	NR	NR	NR	Col	NR	NR		No mutation detected	
47	M	24	NR	NR	NR	NR	NR	Col	NR	NR	Not tested		

* The unit of measurement is year. P, present; MP = mucocutaneous pigmentation; D, diagnosis; F, first polypectomy.

** These three patients had family history of cancer, and their parents who were PJS patients had cancers.

*** There are four novel mutations, which are shown with underlines.

Ad = adopted; BO = bowel obstruction; CE = capsule endoscopy; Col = colonoscopy; CRC = colorectal cancer; Dia = diarrhea; EGD = esophagogastroduodenoscopy; Ent = enteroscopy; FH = family history; G = gastroduodenal; Hemate = hematemesis; Hemato = hematochezia; Int = Intussusception; Lap = Laparotomy; OMC = ovarian mucinous carcinoma; MP = mucocutaneous pigmentation; NA = not available; NR = not recorded; PJS;. = Peutz-Jeghers syndrome; PRP = prolapsed rectal polyp; SB = small bowel; SBC = small bowel cancer; SC = Sertoli cell tumor; Sto = stomachache; Vom = vomiting.

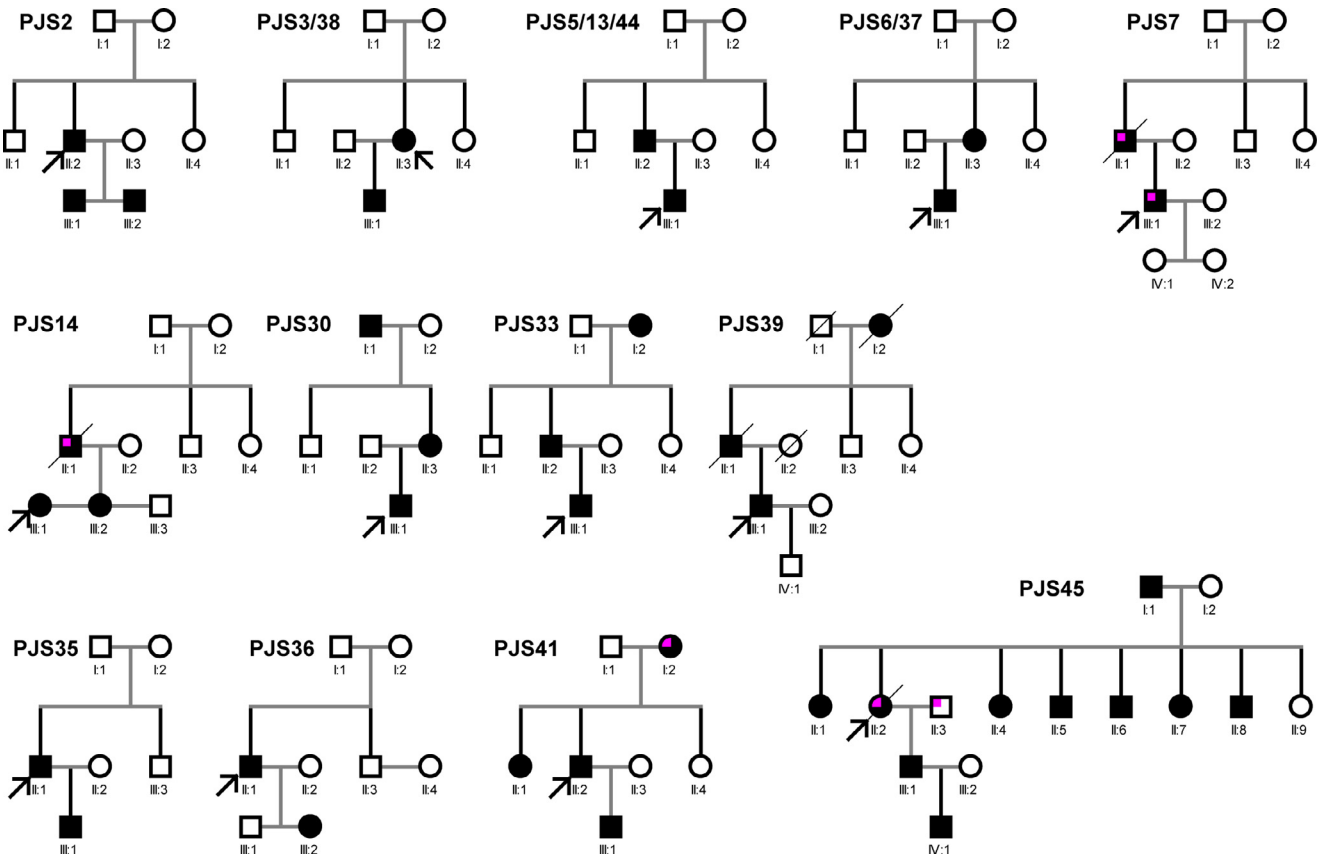

Fig. 1 Genogram of PJS probands with a family history of PJS. Pedigrees of the 17 PJS cases with a positive family history. Roman numerals indicate generations and Arabic numbers indicate individuals. Squares = males, circles = females. Affected individuals are denoted by solid symbols and unaffected individuals are denoted by open symbols. Individuals with cancer history are denoted using a pink label in the upper left quadrant of the symbols. The probands are indicated by arrows. PJS, Peutz-Jeghers syndrome.

Table 3 Information of mutation detection studies in PJS probands.

Author	Year	Country	N*	PCR**		MLPA**		TotalDR (%)	Journal
				N	DR (%)	*N*	DR (%)		
Aretz	2005	Germany	71	37	52.1	17	23.9	76.0	Hum Mutat
Chow	2006	Australia	33	14	42.4	10	30.3	72.7	Clin Genet
Hearle	2006	UK	38	19	50.0	6	15.8	65.8	J Med Genet
Volikos	2006	Europe	76	48	63.2	11	14.5	77.6	J Med Genet
Yang	2010	Korea	17	5	29.4	6	35.3	64.7	Dig Dis Sci***
Orellana	2013	Chile	8	3	37.5	4	50.0	87.5	Clin Genet
Wang	2014	China	52	27	51.9	8	15.4	67.3	Hum Mutat
Zheng	2017	China	11	4	36.4	5	45.5	81.8	J Pediatr Gastroenterol Nutr***
Zhao	2018	China	34	23	67.6	2	5.9	73.5	–

* *N*, number of PJS probands.
** PCR, polymerase chain reaction; MLPA, multiple ligation-dependent probe amplification; DR, detection rate.
*** The probands are children.

dysplasia. However, Hearle's study [17], comprising 419 cases and 297 documented mutations, which is the largest one on this topic, reported that the type or site of *STK11* mutation did not significantly influence cancer risk. Surprisingly, in our study, we noticed a significantly higher frequency of splicing errors in *STK11* in our cohort than in the HGMD (9/25 *vs.* 40/413, $\chi^2 = 16.429$, $p < 0.01$). However, we did not find any association between splicing errors and severe surgical complications or cancers (Table S4 and S5).

In summary, we validated the effectiveness of the combined strategy, consisting of Sanger sequencing and MLPA, for *STK11* mutation detection, with a total detection of 73.5%,

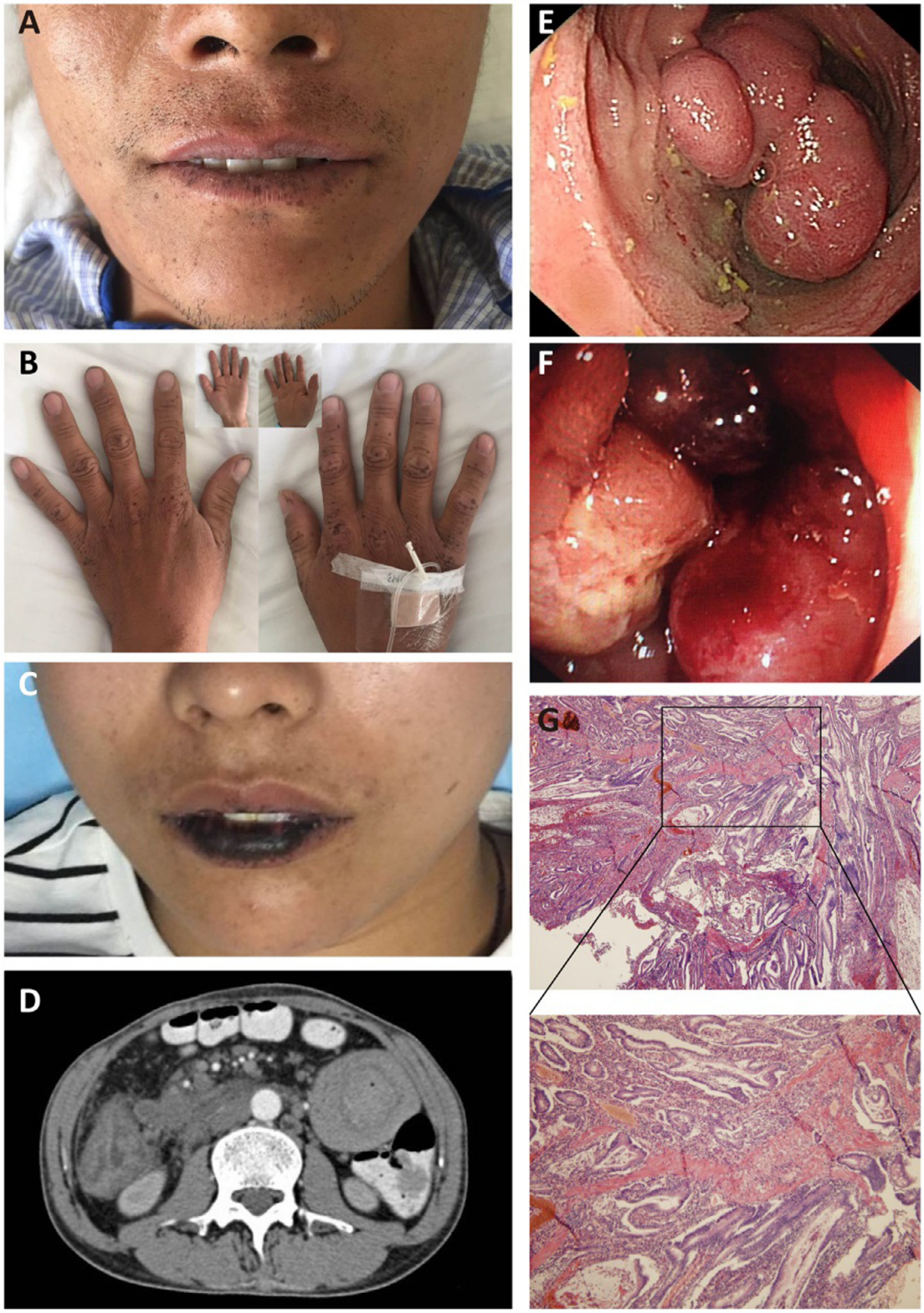

Fig. 2 Clinicopathological feature of patients with PJS patients. (A) and (B) Melanin pigmentation spots of the lips and fingers in patient II: two in PJS13 family. (C) Melanin pigmentation spots of the lips in patient III: one in PJS44 family, which is supernormal obvious. **(**D) Abdominal computed tomography scan of patient III: One in PJS07 showed intussusception in the left abdomen. (E) Endoscopic view of a jejunal giant polyp with a diameter of 5.5 cm in patient PJS02 who was only 4 years old. **(**F) Endoscopic view of the giant polyp in patient PJS07, which already showed tumorigenesis. **(**G) Hematoxylin and eosin-stained tissue slices of the polyp specimens in patient PJS12, confirmed as hamartomatous. Upper, × 40 magnification; lower, × 100 magnification. PJS, Peutz-Jeghers syndrome.

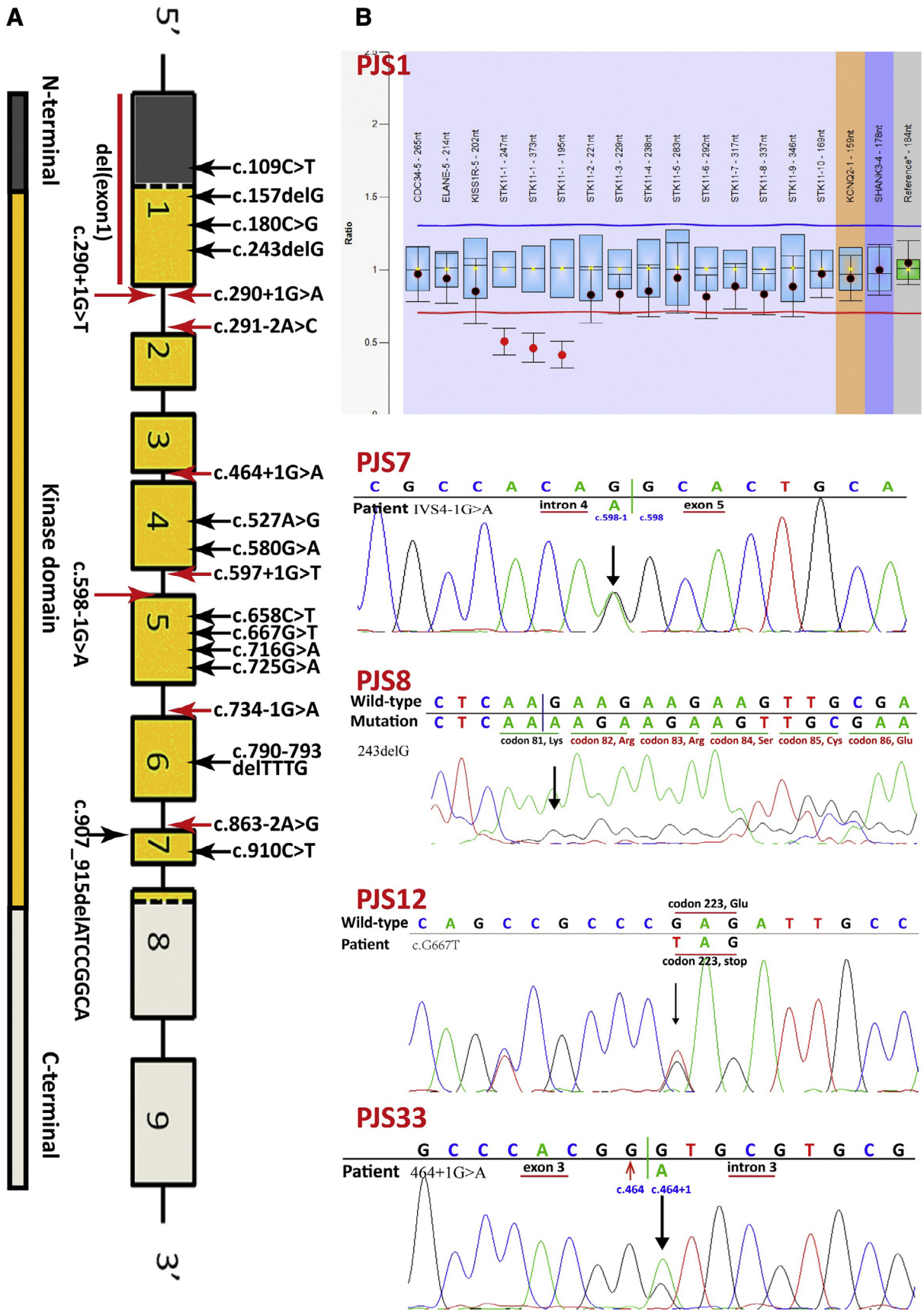

Fig. 3 Distribution of the 25 *STK11* mutations detected (A) and the electropherograms of the four novel mutations, together with MLPA analysis of the del(exon1) (B). MLPA, multiple ligation-dependent probe amplification.

and noticed significantly more splicing errors in this Chinese Han cohort than that in the universal database HGMD. Although no association between the occurrence of severe complications or cancers and certain risk factors was found, we still believe in early screening in patients with PJS patients and the wider use of DBE in PJS surveillance.

Funding

This work was supported by the National Natural Science Foundation of China [Grant number 81500490]; Application Research of Capital Clinical Character [Grant number Z151100004015215]; the Annual Project of Airforce General Hospital [Grant numbers KZ2015026, KZ2016021]; and the National Key R&D Program of China [Grant number 2017YFC1308800].

Informed consent

Written informed consent was obtained from adult participants and from the legal guardians of child participants in this study.

Conflict of interests

The authors declare that they have no competing interests.

Acknowledgments

We thank the subjects for their participation. We very much appreciate the kind help of Dr. Wen-Sheng Lin and Dr. Hong-Yu Cheng's with the pathological and endoscopic images of the polyps.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2018.11.008.

References

[1] Giardiello FM, Brensinger JD, Tersmette AC, et al. Very high risk of cancer in familial Peutz-Jeghers syndrome. Gastroenterology 2000;119:1447–53.
[2] Boardman LA, Thibodeau SN, Schaid DJ, et al. Increased risk for cancer in patients with the Peutz-Jeghers syndrome. Ann Intern Med 1998;128:896–9.
[3] Beggs AD, Latchford AR, Vasen HF, et al. Peutz-Jeghers syndrome: a systematic review and recommendations for management. Gut 2010;59:975–86.
[4] Jenne DE, Reimann H, Nezu J, et al. Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. Nat Genet 1998;18:38–43.
[5] Hemminki A, Markie D, Tomlinson I, et al. A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. Nature 1998;391:184–7.
[6] Boudeau J, Baas AF, Deak M, et al. MO25alpha/beta interact with STRADalpha/beta enhancing their ability to bind, activate and localize LKB1 in the cytoplasm. EMBO J 2003;22:5102–14.
[7] Tiainen M, Ylikorkala A, Makela TP. Growth suppression by Lkb1 is mediated by a G(1) cell cycle arrest. Proc Natl Acad Sci USA 1999;96:9248–51.
[8] Karuman P, Gozani O, Odze RD, et al. The Peutz-Jegher gene product LKB1 is a mediator of p53-dependent cell death. Mol Cell 2001;7:1307–19.
[9] Spicer J, Rayter S, Young N, et al. Regulation of the Wnt signalling component PAR1A by the Peutz-Jeghers syndrome kinase LKB1. Oncogene 2003;22:4752–6.
[10] Ossipova O, Bardeesy N, DePinho RA, et al. LKB1 (XEEK1) regulates Wnt signalling in vertebrate development. Nat Cell Biol 2003;5:889–94.
[11] Smith DP, Rayter SI, Niederlander C, et al. LIP1, a cytoplasmic protein functionally linked to the Peutz-Jeghers syndrome kinase LKB1. Hum Mol Genet 2001;10:2869–77.
[12] Bardeesy N, Sinha M, Hezel AF, et al. Loss of the Lkb1 tumour suppressor provokes intestinal polyposis but resistance to transformation. Nature 2002;419:162–7.
[13] Watts JL, Morton DG, Bestman J, et al. The C. elegans par-4 gene encodes a putative serine-threonine kinase required for establishing embryonic asymmetry. Development 2000;127:1467–75.
[14] Boudeau J, Sapkota G, Alessi DR. LKB1, a protein kinase regulating cell proliferation and polarity. FEBS Lett 2003;546:159–65.
[15] Martin SG, St Johnston D. A role for Drosophila LKB1 in anterior-posterior axis formation and epithelial polarity. Nature 2003;421:379–84.
[16] Baas AF, Kuipers J, van der Wel NN, et al. Complete polarization of single intestinal epithelial cells upon activation of LKB1 by STRAD. Cell 2004;116:457–66.
[17] Hearle N, Schumacher V, Menko FH, et al. Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. Clin Cancer Res 2006;12:3209–15.
[18] Alhopuro P, Phichith D, Tuupanen S, et al. Unregulated smooth–muscle myosin in human intestinal neoplasia. Proc Natl Acad Sci USA 2008;105:5513–18.
[19] Orellana P, Lopez-Kostner F, Heine C, et al. Large deletions and splicing-site mutations in the STK11 gene in Peutz-Jeghers Chilean families. Clin Genet 2013;83:365–9.
[20] Yang HR, Ko JS, Seo JK. Germline mutation analysis of STK11 gene using direct sequencing and multiplex ligation-dependent probe amplification assay in Korean children with Peutz-Jeghers syndrome. Dig Dis Sci 2010;55:3458–65.
[21] Volikos E, Robinson J, Aittomaki K, et al. LKB1 exonic and whole gene deletions are a common cause of Peutz-Jeghers syndrome. J Med Genet 2006;43:e18.
[22] Hearle NC, Rudd MF, Lim W, et al. Exonic STK11 deletions are not a rare cause of Peutz-Jeghers syndrome. J Med Genet 2006;43:e15.
[23] Chow E, Meldrum CJ, Crooks R, et al. An updated mutation spectrum in an Australian series of PJS patients provides further evidence for only one gene locus. Clin Genet 2006;70:409–14.
[24] Aretz S, Stienen D, Uhlhaas S, et al. High proportion of large genomic STK11 deletions in Peutz-Jeghers syndrome. Hum Mutat 2005;26:513–19.
[25] Zheng B, Wang C, Jia Z, et al. A clinical and molecular genetic study in 11 Chinese children with Peutz-Jeghers syndrome. J Pediatr Gastroenterol Nutr 2017;64:559–64.
[26] Wang Z, Wu B, Mosig RA, et al. STK11 domain XI mutations: candidate genetic drivers leading to the development of dysplastic polyps in Peutz-Jeghers syndrome. Hum Mutat 2014;35:851–8.
[27] Aaltonen LA, Hamilton SR. Pathology and genetics of tumours of the digestive system 2010.
[28] Zhao ZY, Jiang YL, Li BR, et al. A 23-nucleotide deletion in

STK11 gene causes Peutz-Jeghers syndrome and malignancy in a chinese patient without a positive family history. Dig Dis Sci 2017;62:3014–20.
[29] Jiang YL, Zhao ZY, Li BR, et al. The altered activity of P53 signaling pathway by STK11 gene mutations and its cancer phenotype in Peutz-Jeghers syndrome. BMC Med Genet 2018;19:141.
[30] Mao X, Zhang Y, Wang H, et al. Mutations of the STK11 and FHIT genes among patients with Peutz-Jeghers syndrome. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2016;33:186–90.
[31] Mao X, Zhang Y, Mao G, et al. STK11 gene mutations in patients with Peutz-Jeghers syndrome. World Chin J Digestol 2015;23:332.
[32] Rufener SL, Koujok K, McKenna BJ, et al. Small bowel intussusception secondary to Peutz-Jeghers polyp. Radiographics 2008;28:284–8.
[33] Harned RK, Buck JL, Sobin LH. The hamartomatous polyposis syndromes: clinical and radiologic features. AJR Am J Roentgenol 1995;164:565–71.
[34] Latchford A, Greenhalf W, Vitone LJ, et al. Peutz-Jeghers syndrome and screening for pancreatic cancer. Br J Surg 2006;93:1446–55.
[35] van Lier MG, Wagner A, Mathus-Vliegen EM, et al. High cancer risk in Peutz-Jeghers syndrome: a systematic review and surveillance recommendations. Am J Gastroenterol 2010;105:1258–64 author reply 1265.
[36] Belsha D, Urs A, Attard T, et al. Effectiveness of double-balloon enteroscopy-facilitated polypectomy in pediatric patients With Peutz-Jeghers syndrome. J Pediatr Gastroenterol Nutr 2017;65:500–2.
[37] Stenson PD, Mort M, Ball EV, et al. The human gene mutation database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next–generation sequencing studies. Hum Genet 2017;136:665–77.
[38] Taylor CF, Charlton RS, Burn J, et al. Genomic deletions in MSH2 or MLH1 are a frequent cause of hereditary non-polyposis colorectal cancer: identification of novel and recurrent deletions by MLPA. Hum Mutat 2003;22:428–33.
[39] Michils G, Tejpar S, Thoelen R, et al. Large deletions of the APC gene in 15% of mutation-negative patients with classical polyposis (FAP): a Belgian study. Hum Mutat 2005;25: 125–134.
[40] Lim W, Hearle N, Shah B, et al. Further observations on LKB1/STK11 status and cancer risk in Peutz-Jeghers syndrome. Br J Cancer 2003;89:308–13.
[41] Olschwang S, Boisson C, Thomas G. Peutz-Jeghers families unlinked to STK11/LKB1 gene mutations are highly predisposed to primitive biliary adenocarcinoma. J Med Genet 2001;38:356–60.
[42] Chiang JM, Chen TC. Clinical manifestations and STK11 germline mutations in Taiwanese patients with Peutz-Jeghers syndrome. Asian J Surg 2017.
[43] van Lier MG, Westerman AM, Wagner A, et al. High cancer risk and increased mortality in patients with Peutz-Jeghers syndrome. Gut 2011;60:141–7.
[44] Mehenni H, Resta N, Guanti G, et al. Molecular and clinical characteristics in 46 families affected with Peutz-Jeghers syndrome. Dig Dis Sci 2007;52:1924–33.
[45] Mehenni H, Resta N, Park JG, et al. Cancer risks in LKB1 germline mutation carriers. Gut 2006;55:984–90.
[46] Schumacher V, Vogel T, Leube B, et al. STK11 genotyping and cancer risk in Peutz-Jeghers syndrome. J Med Genet 2005;42:428–35.
[47] Lim W, Olschwang S, Keller JJ, et al. Relative frequency and morphology of cancers in STK11 mutation carriers. Gastroenterology 2004;126:1788–94.
[48] Amos CI, Keitheri-Cheteri MB, Sabripour M, et al. Genotype--phenotype correlations in Peutz-Jeghers syndrome. J Med Genet 2004;41:327–33.
[49] Salloch H, Reinacher-Schick A, Schulmann K, et al. Truncating mutations in Peutz-Jeghers syndrome are associated with more polyps, surgical interventions and cancers. Int J Colorectal Dis 2010;25:97–107.