J Formos Med Assoc 2010;109(5):354–361

Contents lists available at ScienceDirect

Journal of the Formosan Medical Association

Journal homepage: http://www.jfma-online.com

Original Article

Clinical and Genetic Analysis of Peutz–Jeghers Syndrome Patients in Taiwan

*Meng-Tzu Weng,[1] Yen-Hsuan Ni,[2] Yi-Nien Su,[3] Jau-Min Wong,[4] Shu-Chen Wei[4]**

Background/Purpose: Peutz–Jeghers syndrome (PJS) is an autosomal dominant inherited disorder that is characterized by intestinal hamartomatous polyps and mucocutaneous pigmentation. Recently, germline mutations in the *LKB1* gene have been reported to underlie PJS. The gene that encodes this serine/threonine kinase is located at chromosome 19p13.3. The aim of this study was to investigate the clinical and genetic characteristics of PJS patients in Taiwan.

Methods: We searched the patient database of the National Taiwan University Hospital, a tertiary medical center in Taiwan, between January 1990 and November 2005. Patients' clinical information, demographic data, endoscopic pictures, and outcome were reviewed and analyzed. After obtaining informed consent, DNA and RNA were extracted from peripheral blood mononuclear cells and the *LKB1* gene was sequenced.

Results: A total of 14 unrelated patients who fulfilled the diagnostic criteria of PJS were included, and seven of them had genetic analysis performed. Mucocutaneous pigmentation was the most frequent presentation. Hamartomas occur most commonly in the small intestine (86%). Frequent abdominal complications include intussusception and gastrointestinal bleeding. Four germline mutations were found (57.1%). Three resulted in stop codons at codon 60, 162 (novel mutation), and 308. The fourth mutation was a missense mutation at codon 239 (novel mutation).

Conclusion: Compared with other countries, PJS patients in Taiwan tended to have more extensive polyps in the gastrointestinal tract, with intussusception being the most common abdominal symptom. Mutations in the *LKB1* gene were identified in 57% of the probands in Taiwan.

Key Words: Peutz–Jeghers syndrome, Taiwan

Peutz–Jeghers syndrome (PJS) is an autosomal dominant disorder that is characterized by multiple hamartomatous polyps of the gastrointestinal (GI) tract and by mucocutaneous pigmentation. Approximately 1/120,000 people have PJS.[1] PJS was first identified by the Dutch physician Peutz in 1921, and the original family was described by Jeghers in 1949.[2] These patients often present in the first decade of life with pigmentation or complications of small bowel polyps, such as obstruction or intussusception. In addition to these typical symptoms, patients often have an increased

[1]Department of Internal Medicine, Far Eastern Memorial Hospital, Departments of [2]Pediatrics, [3]Medical Genetics and [4]Internal Medicine, National Taiwan University Hospital and College of Medicine, Taipei, Taiwan.

Received: April 20, 2009
Revised: June 11, 2009
Accepted: July 22, 2009

***Correspondence to:** Dr Shu-Chen Wei, Department of Internal Medicine, National Taiwan University Hospital and College of Medicine, 7 Chung-Shan South Road, Taipei, Taiwan.
E-mail: shuchenwei@ntu.edu.tw

risk of epithelial malignancy, particularly of the GI tract. Other sites include the pancreas, breast, lung, testes and ovaries (so-called sex cord tumors with annular tubules). The overall incidence of cancer among PJS patients has been estimated to be 15-fold higher than that in the general population.[3]

The predisposing gene for PJS has recently been identified as *LKB1*. This gene maps to chromosome sub-band 19p13.3 by linkage analysis, comparative genomic hybridization, and loss of heterozygosity analysis.[4] The gene is predicted to encode a serine/threonine kinase and has also been referred to as serine/threonine kinase 11 (*STK11*). Human *LKB1* consists of nine coding exons with a 433-amino acid coding sequence and one non-coding exon 10. Codons 50–337 encode the catalytic kinase domain. Truncating germline mutations in *LKB1* have been reported in PJS patients.[4,5] Moreover, PJS polyps have typically shown loss of heterozygosity that results in biallelic loss of *LKB1* function.[4] Therefore, it has been suggested that *LKB1* acts as a tumor suppressor in hamartomatous polyps and other neoplasms of PJS patients.[4]

In this study, we report the clinical characteristics of 14 unrelated PJS patients in Taiwan, as well as the genetic analyses of seven of these patients.

Materials and Methods

Patients

Fourteen patients who were diagnosed with PJS from January 1990 to November 2005 in the National Taiwan University Hospital (a tertiary medical center in Taiwan) were retrospectively reviewed and analyzed. The diagnostic criteria for PJS in this study were as follows: (1) three or more histologically confirmed Peutz–Jeghers polyps; (2) any number of Peutz–Jeghers polyps with a family history of PJS; (3) characteristic, prominent, mucocutaneous pigmentation with a family history of PJS; and (4) any number of Peutz–Jeghers polyps and characteristic, prominent, mucocutaneous pigmentation.[6] After signing the informed consent document for participation in our study, which was approved (9000017996) by the Institutional Review Board of the Ethics Committee of the National Taiwan University Hospital, DNA and RNA were extracted from whole venous blood. To clarify further the identified changes as a polymorphism or mutation, population analysis with genomic DNA from 200 anonymous individuals from the DNA bank of the Department of Medical Genetics of the National Taiwan University Hospital was used as the population control.

DNA and RNA purification

Genomic DNA was purified from whole blood by using the Genomic DNA Mini Kit (Geneaid, Tao-Yuan, Taiwan) according to the manufacturer's instructions. Lymphocytes were isolated from whole blood by using the Ficoll-Paque Plus system (Amersham Pharmacia Biotech, Uppsala, Sweden). Total RNA was isolated using the RNeasy kit (Qiagen, Hilden, Germany), following the manufacturer's instructions.

Polymerase chain reaction (PCR)

PCRs of *LKB1* exons were performed in a 50-μL reaction that contained 10 mmol/L Tris–HCl, pH 8.3, 50 mmol/L KCl, 1.5–4.5 mmol/L $MgCl_2$, 50 mmol/L dNTPs, 0.25 mmol/L of each primer, 100 ng genomic DNA, and 1 U Bio-ThermStar DNA polymerase (Gene Craft GmbH, Münster, Germany). The PCR reaction was initiated with a 10-minute denaturation step at 95°C to activate Bio-ThermStar DNA polymerase. Subsequent denaturing steps included 94°C for 20 seconds and an extension step of 72°C for 45 seconds. Primer pairs and the adjusted temperatures for individual amplicons are given in Table 1.

Reverse-transcriptase PCR (RT-PCR)

Standard random priming methods with Moloney murine leukemia virus RT (Promega, Madison, WI, USA) and RNAse inhibitor (Promega) were used to obtain 20 μL cDNA. RT-PCR was performed in Gene Amp PCR System 2400 (PE Applied Biosystems, Foster City, CA, USA) under the following conditions: 3 μL cDNA, 1 × buffer (with $MgCl_2$),

Table 1. Primers and conditions used for *LKB1* gene analysis

LKB1 gene		Sequence	T_m (°C)	Product size (bp)
genomic DNA				
Exon 1	forward	5′-CGG ACT CAG GGC TGG CGG CG-3′	62	385
	reverse	5′-CAG CAC CGT GAC TGG CCC GGC-3′		
Exon 2	forward	5′-CGT TGG GTC GGC TGA TAC-3′	60	164
	reverse	5′-TCC CAC GGA GGC CCC GCG G-3′		
Exon 3	forward	5′-GGC CTG TGA GTG GGG CCG-3′	66	201
	reverse	5′-GGA GCC TGC CCT GCC TGG, CC-3′		
Exon 4–5	forward	5′-AGG GAG GCC TCG GCC CCA G-3′	66	426
	reverse	5′-GTG TGC GTG TGG TGA GTG C-3′		
Exon 6	forward	5′-CTT GAC TGA CCA CGC CTT TC-3′	60	248
	reverse	5′-ACC TGA CAC CCC CAA CCC TAC-3′		
Exon 7	forward	5′-TGC CCA GCT GAC AGG CTC C-3′	64	136
	reverse	5′-TCC CTG CAG CCT CGG CCC CAC-3′		
Exon 8	forward	5′-ACT GGA CCG CCC TGG TGC CAG-3′	66	295
	reverse	5′-GGA CAT CCT GGC CGA GTC AGC-3′		
Exon 9	forward	5′-GCG CCC CTC AGC TCA GGC CAC-3′	64	349
	reverse	5′-CGG TCA CCA TGA CTG ACT AGC-3′		
cDNA				
Exon 1	forward	5′-GAG AAG GGA AGT CGG AAC A-3′	60	1101
	reverse	5′-CGG CAC CAC AGT CAT GC-3′		
Exon 2	forward	5′-CGG GTA CTT CTG TCA GCT G-3′	62	906
	reverse	5′-GAA GAC TGA GGG CCT GG-3′		

250 μM dNTPs, 0.8 μM reverse and forward primers, 2 U Amplitaq Gold polymerase (PE Applied Biosystems) and water, in a final volume of 50 μL. The RT-PCR products obtained with the primer sets are also listed in Table 1.

Direct sequencing

Sequencing reactions were performed for the PCR products of the *LKB1* gene using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems). Electrophoresis was carried out using a Genetic Analyzer 310 (PE Applied Biosystems) equipped with long-read sequencing capillary and POP-6 sequencing polymer (PE Applied Biosystems). Primers used for direct sequencing reactions were the same as for the PCRs (Table 1).

Results

Clinical features

The clinical phenotypes of the 14 unrelated PJS patients are summarized in Table 2. There were four male and 10 female patients, and symptoms appeared at a median age of 19 years. The mean age at diagnosis was 18.9 years (range, 2–72 years). Family history was positive in six cases (42.9%). All these patients presented with numerous pigmented spots on the lips and the buccal mucosa as the extra-GI tract manifestation. They were also characterized by the presence of multiple GI hamartomatous polyps (93%). Hamartomas occurred most commonly in the small intestine (86%), but also in the stomach (79%), and colon (71%).

Table 2. Clinical characteristics of Peutz–Jeghers patients

Case	Age (yr)/sex	Age at diagnosis (yr)	Pigmentation	Familial	*LKB1* mutation	Polyp location	Clinical history
1	14/F	12	Yes	No	Yes	Stomach, ileum, rectum	Laparotomy, manual reduction and polyp resection, 12-yr-old, intussusception, 3 ileal polyp
2	18/F	11	Yes	No	Yes	Stomach, duodenum, jejunum, rectum	Jejunum resection at 14 yr of age, intussusception, 1 jejunum polyp
3	10/F	2	Yes	No	–	Stomach, jejunum, D-colon	D-colon reduction and surgical polypectomy, 7-yr-old, intussusception, 1 D-colon polyp
4	22/F	13	Yes	Yes	No	Stomach, duodenum, jejunum, ileum, A-colon	Manual reduction and segmental resection of terminal ileum. Two jejunum–jejunum intussusception and ileum–T-colon intussusception, 16-yr-old, several jejunum and ileum polyps Open reduction + segmental ileum resection, ileal–ileal intussusception, 21-yr-old, multiple ileum polyps
5	19/F	15	Yes	No	Yes	Stomach, duodenum, sigmoid colon	Enterotomy at 17 yr of age, duodenal polyps
6	10/F	8	Yes	Yes	–	Jejunum	Not remarkable
7	58/F	36	Yes	No	No	Stomach, duodenum, jejunum, Sigmoid colon	Spontaneous reduction at 57 yr of age, intussusception, duodenum and jejunum polyps s/p endoscopic polypectomy
8	10/M	3	Yes	No	No	Cecum, rectum	Not remarkable
9	26/F	22	Yes	Yes	–	Stomach, duodenum, colon	Small bowel resection at 22 yr of age, intussusception, jejunum polyps
10	42/F	16	Yes	Yes	–	Stomach, duodenum, rectum	Spontaneous reduction at 35 yr of age, intussusception, duodenum polyps s/p endoscopic resection
11	42/M	28	Yes	Yes	Yes	Stomach, duodenum, jejunum, colon	Small bowel resection at 28 yr of age, intussusception, jejunum polyps. Right hemicolectomy at 38 yr of age, colon cancer, colon polyps
12	74/M	72	Yes	No	–	Stomach	1. Enterotomy at 73 yr of age, 1 gastric cardia polyp 2. Lung cancer
13	23/M	12	Yes	No	–	Stomach, duodenum, jejunum, ileum	Reduction and enterotomy at 12 yr of age, intussusception, 4 stomach polyps, 1 duodenal polyp, 18 jejunal and ileal polyps
14	26/F	14	Yes	Yes	–	Jejunum	Enterotomy at 14 yr of age, 1 jejunal polyp

M = Male; F = female; – = not tested; D-colon = descending colon; A-colon = ascending colon; T-colon = transverse colon.

The most frequent complication in these patients was anemia (78.6%), followed by polyp-related intussusception (64.3%). Seven of the nine patients with polyps (77.8%) received surgery for intussusception, and the others received conservative treatment. Three patients suffered from episodes of profound GI bleeding; two subsided after endoscopic treatment and the third required surgical intervention. One patient developed Dukes' classification B1 colon cancer. Subtotal colectomy was performed and no evidence of recurrence was noted after a 5-year follow-up. Another patient had lung cancer with bone metastasis and was later lost to follow-up. No mortality was noted in these patients from the chart record (Table 3).

Genetic analysis

Mutations were detected in four of the seven probands (57%) subjected to genetic analysis of the *LKB1* gene from genomic DNA. The direct sequencing results are displayed in Figure 1 and summarized in Table 4. Among the four mutations, two nonsense mutations, one frameshift and one missense mutation were identified. Case 2 was a guanine to adenine substitution at base 923 that resulted in a change from tryptophan to a premature stop signal at codon 308 in exon 8. Case 5 was an insertion of cytosine at base 117 that created a frameshift mutation that resulted in a premature stop signal at codon 162. Case 11 was a thymine to guanine substitution at base 180 that resulted in a premature stop signal at codon 60 [TAC (Tyr) to TAG (stop)]. Case 1 was a missense mutation with a thymine to guanine substitution at base 715 that resulted in a tryptophan to glycine change at codon 239 in exon 5. This missense change was not detected in the 200 control DNA samples.

All four mutations were identified by PCR from genomic DNA. Only one mutation was detected by RT-PCR based on cDNA. The RT-PCR product of case 5 was displayed as two fragments of 569 and 1101 bp (Figure 2). The size of the smaller fragment was compatible with the premature stop product, which did not appear in the control samples. However, no premature stop products were found in the RT-PCR analysis of cases 2 and 11. This may be explained by the rapid destruction of the mutated transcripts, which is known as nonsense-mediated mRNA decay.

Table 3. Peutz–Jeghers-syndrome-related complications in 14 patients*

Complications	Cases
Anemia	11 (78.6)
Tarry or bloody stool	3 (21.4)
Polyp-related intussusception	9 (64.3)
Polyp-related bowel obstruction	7 (50.0)
Cancer (Colon, lung)	2 (14.3)
Protein-losing enteropathy	1 (7.1)

**Data presented as n (%).*

Discussion

Although PJS is not a common disease, patients usually have major medical/surgical issues (such as intussusception or GI bleeding) in early life and a higher risk of cancer in later life. It is important

Table 4. Germline mutation identified in the *LKB1* gene

Case	*LKB1* change	Exon	Bases	Codons	Amino acid change
1	TGG → TGG+GGG	5	715	239	W → G (unknown significance)
2	TGG → TGG+TAG	8	923	308	W → stop codon
4	–				
5	Insert C	1	117	39	Stop at codon 162
7	–				
8	–				
11	TAC → TAC+TAG	1	180	60	Y → stop

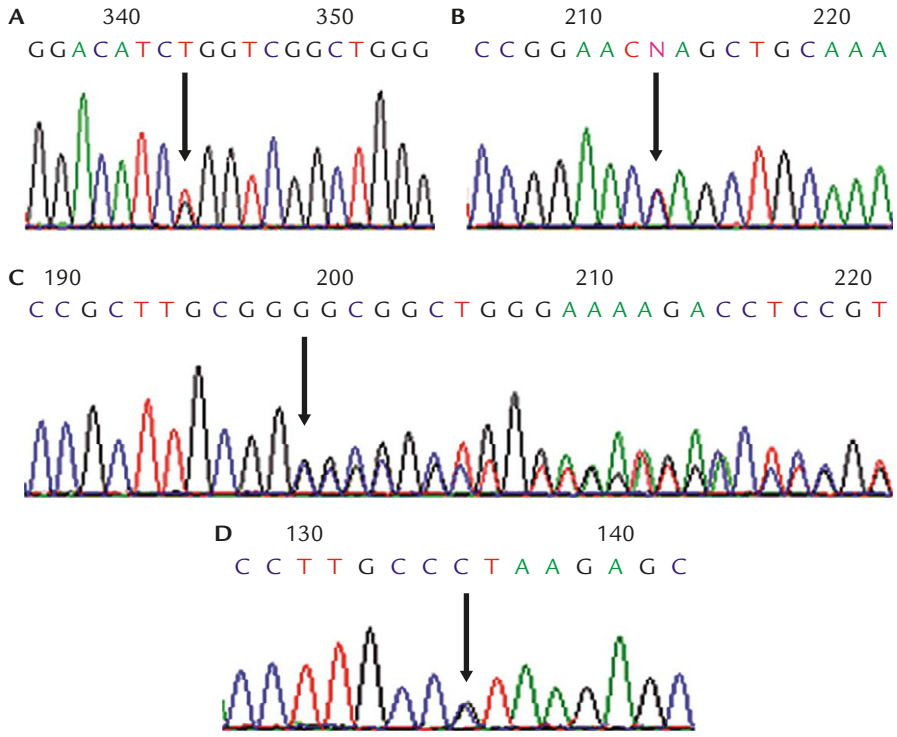

Figure 1. *LKB1* germline mutations in familial and sporadic Peutz–Jeghers patients. Mutations are arrowed. As per standard ABI sequencing analysis format, blue = C, black = G, red = T, and green = A. (A) T715 → G change in case 1 (forward sequence), TGG (W) → TGG (W) + GGG (G); (B) G923 → A transition in case 2 (reverse sequence), TGG (W) → TGG (W) + TAG (stop); (C) single C insertion at base 117 in case 5 (reverse sequence), CGC (R) → CGCC (mutant); (D) C180 → G change in case 11 (reverse sequence), TAC (Y) → TAC (Y) + TAG (stop).

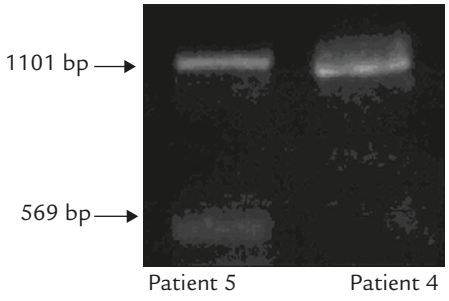

Figure 2. Mutation analysis of the *LKB1* cDNA clone in PJS patients. The heterozygous mutation was detected in cDNA of case 5 displayed as two fragments of 569 and 1101 bp. The cDNA of case 4 was comparable with the normal transcript size (1101 bp).

for the clinician to recognize this syndrome and follow up these patients regularly to decrease and prevent related complications. In our patients, the mean age at diagnosis was 18.9 years, which was earlier than in a previous study, which has suggested that diagnosis is usually made in the second or third decade of life.[7] The earlier diagnosis in our patients might have been related to the more extensive polyps located in the GI tract. Polyps with small bowel, large bowel and stomach involvement were found in 95%, 76% and 64.4% of our patients, respectively. However, polyps with small bowel, large bowel and stomach involvement have been found in 64–96%, 60% and 24–49%, respectively, in other studies.[8–10]

The reported symptoms/signs of PJS include abdominal pain, rectal bleeding, anemia, small intestine intussusception, bowel obstruction, and rectal prolapse.[7] Anemia was the most common complication in our PJS patients (78.6%). Nine of our patients had intussusception, and 7 of them (77%) were surgically treated. This surgical rate was higher than that reported in a Japanese study in which 47% of patients who had intussusception required surgery. This discrepancy could be because our hospital is a tertiary referral medical center in Taiwan, where most of the patients were referred and arrived with more severe disease.

To prevent repeat laparotomy, many studies have used intraoperative endoscopy and endoscopic polypectomy to replace enterotomy. Two of our PJS patients (14%) were complicated with cancer. A dramatically increased relative and absolute risk of GI and extra-GI cancers has been documented in patients with PJS.[3,11–13] To detect GI tract malignancy early, most experts recommend colonoscopy at intervals of 2–3 years, beginning at age 18 years, and upper GI tract surveillance at intervals of 2–3 years from age 25 years. Surveillance for pancreatic cancer with endoscopic or transabdominal ultrasonography should begin at 30 years of age. Biennial mammography beginning at age 20 years is recommended in women.[3]

Our genetic analysis of PJS patients revealed four *LKB1* gene mutations (57%) in seven probands. This mutation rate was comparable to that in a previous study in which germline *LKB1* mutations occurred in 50–75% of PJS patients, by using genomic DNA or cDNA sequencing as a primary screen.[5] Most of the germline mutations detected in PJS were frameshift or nonsense changes that resulted in truncated proteins. Seventy-five percent of the mutations in this study led to truncated mutations (stop codons at 60, 162 and 308) and were located within the catalytic (kinase) domain of *LKB1* (codons 50–337), which suggests that the activity of the serine/threonine kinase was inhibited.

There were two novel mutations identified in our study. The first was discovered in a 19-year-old girl (diagnosed at age 15 years) with a single cytosine insertion at base 117 that caused a frameshift stop at codon 162 and loss of the kinase function. The second was a missense change (tryptophan > glycine) at position 239 in a 14-year-old girl. Exchange of tryptophan (the largest amino acid) to glycine (the smallest amino acid) has the potential to alter the quaternary structure of the expressed protein and lead to functional change, such as interruption of phosphorylation and post-translational events that are critical for STK11 activity. In addition, this transition was not detected in 200 unrelated healthy individuals, thus polymorphism was not favored. However, the mechanism that underlies the region of this mutation is unclear and the cause of clinical significance needs to be clarified by further study.

Two other mutations have been reported previously. The nonsense mutation in case 2 (codon 308) has been described by Ylikorkala et al.[14] However, the phenotype of these two patients cannot be compared since no clinical information was mentioned in the study. Case 11 had a thymine to guanine substitution at base 180 that resulted in a premature stop signal at codon 60 [TAC (Tyr) to TAG (stop)], and developed colon cancer. This mutation has been reported twice previously, although whether or not those patients had cancer was not recorded.[5]

By evaluating the correlation between mutation status and clinical characteristics, Amos et al discovered that 27% of the detected pathogenic mutations in *LKB1* were missense mutations. These patients typically have a later time of onset of PJS symptoms. Truncation of the encoded *STK11* gene leads a significantly earlier age of onset of PJS symptoms than in patients who have a missense mutation or no detectable mutation of *STK11*.[15] However, we could not reach a similar conclusion because we do not yet have enough cases.

In conclusion, intussusception, anemia and cancer were common clinical problems in our PJS patients. *LKB1* germline mutations were detected in 57% of Taiwanese PJS patients, which confirmed that *LKB1* is the major gene responsible for PJS in the Taiwanese population. Combination of genetic analysis to achieve early diagnosis of PJS with a regular screening program to decrease polyp-related complications and the effects of cancer is important in the treatment and follow-up of these patients.

References

1. Lindor NM, Greene MH. The concise handbook of family cancer syndromes. Mayo Familial Cancer Program. *J Natl Cancer Inst* 1998;90:1039–71.
2. Jeghers H, Mc KV, Katz KH. Generalized intestinal polyposis and melanin spots of the oral mucosa, lips and digits;

a syndrome of diagnostic significance. *N Engl J Med* 1949; 241:993.
3. Giardiello FM, Brensinger JD, Tersmette AC, et al. Very high risk of cancer in familial Peutz–Jeghers syndrome. *Gastroenterology* 2000;119:1447–53.
4. Hemminki A, Tomlinson I, Markie D, et al. Localization of a susceptibility locus for Peutz–Jeghers syndrome to 19p using comparative genomic hybridization and targeted linkage analysis. *Nat Genet* 1997;15:87–90.
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. Aaltonen L JH, Gruber S. Peutz–Jehgers syndrome. In: Hamilton S, Aaltonen L, eds. *Tumours of the Digestive System*. Lyon: IACR, 2000:74–6.
7. McGarrity TJ, Amos C. Peutz–Jeghers syndrome: clinicopathology and molecular alterations. *Cell Mol Life Sci* 2006;63:2135–44.
8. Choi HS, Park YJ, Youk EG, et al. Clinical characteristics of Peutz–Jeghers syndrome in Korean polyposis patients. *Int J Colorectal Dis* 2000;15:35–8.
9. Dai YC, Song YG, Xiao B, et al. Clinical classification of Peutz–Jeghers syndrome. *Nan Fang Yi Ke Da Xue Xue Bao* 2006;26:79–81. [In Chinese]
10. Utsunomiya J, Gocho H, Miyanaga T, et al. Peutz–Jeghers syndrome: its natural course and management. *Johns Hopkins Med J* 1975;136:71–82.
11. Giardiello FM, Welsh SB, Hamilton SR, et al. Increased risk of cancer in the Peutz–Jeghers syndrome. *N Engl J Med* 1987;316:1511–4.
12. Hizawa K, Iida M, Matsumoto T, et al. Cancer in Peutz–Jeghers syndrome. *Cancer* 1993;72:2777–81.
13. Yoon KA, Ku JL, Choi HS, et al. Germline mutations of the STK11 gene in Korean Peutz–Jeghers syndrome patients. *Br J Cancer* 2000;82:1403–6.
14. Ylikorkala A, Avizienyte E, Tomlinson IP, et al. Mutations and impaired function of *LKB1* in familial and non-familial Peutz–Jeghers syndrome and a sporadic testicular cancer. *Hum Mol Genet* 1999;8:45–51.
15. Amos CI, Keitheri-Cheteri MB, Sabripour M, et al. Genotype–phenotype correlations in Peutz–Jeghers syndrome. *J Med Genet* 2004;41:327–33.