Clinical characteristics and genetic analysis of gene mutations in a Chinese pedigree with Peutz-Jeghers syndrome

Yudian Qiu | Tao Xuan | Mujun Yin | Zhidong Gao | Peng Guo | Xi Chen | Yingjiang Ye | Zhanlong Shen

1Peking University People’s Hospital, Beijing, China
2Geneplus Co. Ltd, Beijing, China
3Department of Gastroenterological Surgery, Peking University People’s Hospital, Beijing, China

Correspondence
Zhanlong Shen, Department of Gastroenterological Surgery, Peking University People’s Hospital, Beijing, China.
Email: shenlongz1977@126.com

Funding information
National Natural Science Foundation of China, Grant/Award Number: 81672375

Key Clinical Message
The genome-wide sequencing information of PJS is still lacking. Our result demonstrates that c.862+2T>C variant on STK11 as an important foundation of molecular mechanism in this familial PJS. Variants in KDR and MLL3 may play important roles in the initiation and development of this familial PJS polyps.

KEYWORDS
germline variants, high-throughput sequencing, Peutz-Jeghers syndrome, somatic gene variants, STK11

1 | INTRODUCTION

Peutz-Jeghers syndrome (PJS) is a rare autosomal dominant-genetic disease, the incidence of which has been estimated to be approximately 1 in 50 000-200 000 births, it is characterized by the mucocutaneous melanin pigmentation, multiple gastrointestinal polyps, and elevated risk for cancer, involving benign and malignant tumors of multiple organs both in the gastrointestinal tract and extra-gastrointestinal sites such as lungs, breasts, ovaries, and uterine cervixes. Recent studies suggest that the mutations of the gene STK11 located on the short arm of chromosome 19(19p13.3) are important molecular bases for the pathogenesis of PJS. Multiple germline mutations and somatic mutations have been identified in PJS patients, yet similar kind of research in this area is still lacking. In this research, we study the genetic information in the patient and her father’s peripheral blood cells to screen for the existence of germline genetic mutations using high-throughput sequencing technology and detect the somatic gene mutation in the patient’s polyp specimens. Our study aims to find types of STK11 variant in our patient and detect any other types of variant. Our results could help this family avoid a high risk of having a child with PJS by preimplantation genetic testing, and would be helpful in predicting cancer risks and provide some meaningful guidance for the clinical work.

2 | CASE PRESENTATION

A 26-year-old young woman presented to our emergency department with chief complaint of abdominal pain with distension, vomiting with defecation stopped for 17 hours. She
reported a history of intestine intussusception that had been cured 15 years ago. Pigmented macules over the lower lip, bilateral buccal mucosa, and digits with pale conjunctiva and hyponychiums were found on physical examination (Figure 1A,B,C). No significant expansion of intestines, no organ injury, or liquid gas plane was seen in the Abdominal plain film (Figure 1D), yet small intestine-to-small intestine intussusception led by a polyp was advised in the computed tomography (CT) (Figure 1E, arrows). The expansion and edema of the small intestine as well as multiple localized intraluminal polyp lesions existed. G. Image from transition part of edema intestine and normal intestine during the surgery. H. Image from surgical specimen from intestine resection with the lumen opened displaying the inner bowel mucosa and mass. I. Image from histopathological evaluation of the biopsy specimen obtained from the lesion suggests multiple regional adenomatous hyperplasia and interstitial focal lymphocyte infiltration, smooth muscle bundle can be seen around the gland.

FIGURE 1 Pictures in patient’s physical examination, the abdominal plain film, and contrast-enhanced abdominal CT, pictures during the surgery and resected intestine, histopathological image of the patient. A-C, Digits, oral mucocutaneous and lower lip pigmentation characteristic of PJS patient. D. Image from abdominal plain film demonstrating no significant expansion of intestinals, no organ injury or liquid gas plane. E, Image from contrast-enhanced abdominal CT scan confirming small intestine-to-small intestine intussusception leaded by a polyp. F, Expansion and edema of the small intestine as well as multiple localized intraluminal polyp lesions existed. G, Image from transition part of edema intestine and normal intestine during the surgery. H, Image from surgical specimen from intestine resection with the lumen opened displaying the inner bowel mucosa and mass. I. Image from histopathological evaluation of the biopsy specimen obtained from the lesion suggests multiple regional adenomatous hyperplasia and interstitial focal lymphocyte infiltration, smooth muscle bundle can be seen around the gland.

Ten milliliter blood was taken from both the patient and her father after their approvals. The blood samples, together with the samples of biopsy, were subsequently tested with the high-throughput sequencing, the somatic sequencing NGS results were derived from one polyp. The total DNAs of polyp tissues and peripheral blood cells were extracted according to standard protocols and were sheared to 300-bp fragments with a Covaris S2 ultrasonicator. Indexed Illumina NGS libraries from tissues and the blood cells were prepared using KAPA Library Preparation Kit (Kapa Biosystems,
Wilmington, MA, USA). Target enrichment was performed with a custom SeqCap EZ Library (Roche NimbleGen, Madison, WI, USA).

The capture probe used for detecting germline mutation was designed based on genomic regions of 58 genes, and the other probe used for detecting somatic mutation was designed based on genomic regions of 1021 genes most frequently mutated in colon tumor and other common solid tumors. The 58 genes and 1021 genes we studied are commercial sets, part of the OncoH and OncoD product separately. Capture hybridization was carried out according to the manufacturer’s protocol. Following hybrid selection, the captured DNA fragments were amplified and then pooled to generate several multiplex libraries.

Sequencing was carried out using Illumina 2 × 75 bp paired-end reads on an Illumina HiSeq 2500 instrument according to the manufacturer’s recommendations using TruSeq PE Cluster Generation Kit v3 and the TruSeq SBS Kit v3 (Illumina, San Diego, CA, USA).

After removing terminal adaptor sequences and low-quality data, the reads were mapped to the reference human genome and aligned. GATK (https://www.broadinstitute.org/gatk/, The Genome Analysis Toolkit) and MuTect were employed to call somatic small insertions and deletions (indels) and single nucleotide variants (SNVs) by filtering blood cell sequencing data. Contra was used to detect copy number variations, and local algorithm was used to detect cancer-associated structure variations.

4 | RESULTS

4.1 | Findings in the operation

The emergency surgery revealed an intussusception that was 2.6 m from the ileocecal valve, which was caused by an approximately 1 cm × 2 cm sized pedunculated polyp, the proximal intestines were dilated and edematous with multiple polyps of various sizes explored outside the intestinal duct. The highest position of the polyps reached 40 cm from the treze ligament. Five of the polyps were larger than 1 cm, the largest reached 3 cm × 4 cm. No ischemic necrosis was seen (Figure 1G). We performed partial resection of small intestine and intestinal anastomosis as well as the local resection to remove the adhesions and reduce the intussusception. A 30 cm length of intestinal tube with three big polyps (≥1 cm) and dozens of small polyps (<1 cm), as well as two separate large polyps, was resected. The specimen is shown (Figure 1H). The operation went smoothly, and the patient was sent to the intensive care unit for recovery after the surgery.

4.2 | Histopathological results

Histopathological evaluation of the biopsy specimens obtained from the lesion suggested multiple regional adenomatous hyperplasia and interstitial focal lymphocyte infiltration. Smooth muscle bundle can be seen around the gland (Figure 1I), which corresponds to the typical characteristics of Peutz-Jeghers type hamartomatous polyps.

4.3 | Sequencing results

Among 58 types of germline genetic mutations we detected, only STK11 gene variant was detected in peripheral blood samples of the patient and her father. One single-base substitution type of heterozygous variant (c.862+2T>C) was found in the patient and her father (Table 1). Somatic gene mutations of patient’s polyps tissue were detected, using the DNA extracted from the patient’s blood cells as the control. Only two somatic variants were found: KDR (c.1699G>A, p.V567M) and MLL3 (c.4035G>T, p.K1345N). The frequencies of the two variants were 15.31% and 14.47%, respectively (Table 2).

5 | DISCUSSION

Many literatures have reported that variants in STK11 are important molecular basis for the pathogenesis in PJS patients, more than 300 STK11 pathogenic variants have been reported according to the Human Gene Mutation Database. The STK11 gene variant we detected was located on site c.862+2T>C, it is a kind of missense mutation which was once reported in a PJS patient, and recorded in the ZJU-CGGM database (http://www.genomed.org/lov2/variants.php?select_db=STK11&action=view&view=0002938%2C0000453%2C0). No somatic mutations, copy number variation (CNV), or loss of heterozygosity (LOH) of STK11 were detected other than c.862+2T>C in our assays. No structural variation (SV) or copy number variation of other tested genes was detected either. We believe that site c.862+2T>C variant of STK11 gene is an important molecular basis for pathogenesis in the patient and her father.

| TABLE 1 | Germline genetic mutations of blood cells |
|----------|------------------------------------------|
| Start    | End        | Gene | cHGVS     | pHGVS | Function | Frequency (%) | Homo/Heter |
| Patient  | 1 221 340 | 1 221 341 | STK11    | c.862+2T>C | -     | Splice-5   | 49          | Heter        |
| Patient’s father | 1 221 340 | 1 221 341 | STK11    | c.862+2T>C | -     | Splice-5   | 47          | Heter        |

cHGVS, nucleic acid Human Genome Variation Society; Heter, heterogeneous; Homo, homogeneous; pHGVS, protein Human Genome Variation Society.
KDR plays a key role in angiogenesis, and is closely related to the recurrence, metastasis and prognosis of a variety of tumors. The expression of VEGF/KDR is closely related to the metastasis of colorectal cancer. MLL3 catalyzes the monomethylation of large amounts of histone H3K4 and is known as a tumor suppressor gene. The mutations of MLL3 were found in a variety of tumors such as breast cancer, rectal cancer, retinoblastoma, gastric cancer, bladder cancer, and liver cancer. The mutations of MLL3 in these tissues result in a declined methylation level of H3K4. This reduces the activation of the enhancer, resulting in the inactivation of some kinds of tumor suppressor genes, involving in tumorigenesis. Vogelstein et al revealed that colorectal cancers result from the sequential accumulation of mutations in oncogenes and tumor suppressor genes, mutations in at least four or five genes are required to produce a malignant tumor, the total accumulation of changes is responsible for determining the tumor’s biologic properties. So the mutations in KDR and MLL3 are not enough to enable the patient progressed to the early stages of tumor development up to now.

PJS can cause chronic bleeding and anemia as well as recurrent obstruction and intussusception. Individuals with PJS are at increased risk for various kinds of tumors, especially epithelial neoplasias. A large number of the previous articles on PJS gene analysis only use techniques such as single-gene testing and PCR, while genome-wide sequencing information is still lacking. In addition, STK11 is complex and still being clarified, though the STK11 mutation is "pathogenic mutation," no clear genotype-phenotype correlation has been demonstrated in PJS, studies of PJS families looking for other PJS locus suggested that in some the 19p13.3 locus was not involved in PJS. This has raised the possibility of genetic heterogeneity. In this study, we performed a high-throughput sequencing on the patient’s and her father’s peripheral blood cell samples to detect the germline mutations, and found the hereditary heterozygous variant of STK11 gene, site c.862+2T>C. The variants of somatic cells in polyp tissues were also detected, variant frequencies of KDR and MLL3 were 15.31% and 14.47%, respectively. We believe that site c.862+2T>C variant in the STK11 gene is an important foundation of molecular mechanism in this familial PJS. This is consistent with the findings of previous studies. However, we did not detect any variants in the STK11 gene except c.862+2T>C in the patient’s polyp tissues. The CNV and LOH of the STK11 gene were not detected either. At the same time, our detection of KDR and MLL3 gene variants in the patient’s polyp tissues may also be involved in the formation and development of polyps, but the patient did not progress to the early stages of tumor development at present.

6 | CONCLUSION

Our result demonstrates that c.862+2T>C variant on STK11 as an important foundation of molecular mechanism in this familial PJS. Variants in KDR and MLL3 may play important roles in the initiation and development of the polyps, indicating a high risk of cancer. Our findings could help this family avoid a high risk of having a child with PJS by preimplantation genetic testing and could help clinicians better understand and provide better clinical surveillance and therapies for PJS patients.

ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation of China, Grant number: 81672375. We would like to express our gratitude to the patient and her father who participated in this study. We also thank Geneplus Co., Ltd for the technical support in our study.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

ZS and YQ: designed the report; ZS, YY, YQ, ZG and PG: performed the surgery; MY and XC: performed the pathological diagnosis; YQ and TX: analyzed the data and wrote the manuscript, All authors read and approved the final manuscript.

Consent for publication: The patient and her father included in this study allowed this paper to include some information of their disease for publication. Written informed consent for publication of the clinical details was obtained.
REFERENCES

1. 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;55:3458-3465.
2. 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.
3. Aretz S, Stienen D, Uhlhaas S, et al. High proportion of large genomic STK11 deletions in Peutz-Jeghers syndrome. *Hum Mutat*. 2005;26:513-519.
4. Olschwang S, Markie D, Seal S, et al. Peutz-Jeghers disease: most, but not all, families are compatible with linkage to 19p13.3. *J Med Genet*. 1998;35(1):42-44.
5. Buchet-Poyau K, Mehenni H, Radhakrishna U, Antonarakis Se. Search for the second Peutz-Jeghers syndrome locus: exclusion of the STK13, PRKCG, KLK10, and PSCD2 genes on chromosome 19 and the STK11IP gene on chromosome 2. *Cytogenet Genome Res*. 2002;97:171-178.
6. Alhopuro P, Phichith D, Tuupanen S, et al. Unregulated smooth-muscle myosin in human intestinal neoplasia. *Proc Natl Acad Sci USA*. 2008;105:5513-5518.
7. Beggs AD, Latchford AR, Vasen H, et al. Peutz-Jeghers syndrome: a systematic review and recommendations for management. *Gut*. 2010;59:975-986.
8. Hearle N, Schumacher V, Menko FH, et al. Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. *Clin Cancer Res*. 2006;12:3209-3215.
9. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z. Vascular endothelial growth factor (vEGF) and its receptors. *FASEB J*. 1999;13:9-22.
10. Takahashi Y, Kitadai Y, Bucana CD, et al. Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. *Cancer Res*. 1995;55:3964-3968.
11. Wang XX, Fu L, Li X, et al. Somatic mutations of the mixed-lineage leukemia 3 (MLL3) gene in primary breast cancers. *Pathol Oncol Res*. 2011;17:429-433.
12. Li B, Liu H-Y, Guo S-H, Sun P, Gong F-M, Jia B-Q. MLL3 genetic variants affect risk of gastric cancer in the chinese han population. *Asian Pac J Cancer Prev*. 2013;14:4239-4242.
13. Raizis AM, Van Mater D, Aaltonen LA, et al. Trilateral retinoblastoma in a patient with peutz-jeghers syndrome. *Am J Med Genet A*. 2013;161:1096-1100.
14. Lawrence MS, Stojanov P, Mermel CH, et al. Discovery and saturation analysis of cancer genes across 21 tumour types. *Nature*. 2014;505:495-501.
15. Fujimoto A, Totoki Y, Abe T, et al. Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. *Nat Genet*. 2012;44:760-764.
16. Cho KR, Vogelstein B. Genetic alterations in the adenoma–carcinoma sequence. *Cancer*. 1992;70(6 Suppl):1727.
17. Cosme A, Ojeda E, San Vicente MT, et al. Peutz-Jeghers syndrome associated with multiple epithelial tumors. *Gastroenterol Hepatol*. 2001;24:495-499.

How to cite this article: Qiu Y, Xuan T, Yin M, et al. Clinical characteristics and genetic analysis of gene mutations in a Chinese pedigree with Peutz-Jeghers syndrome. *Clin Case Rep*. 2019;7:735-739. [https://doi.org/10.1002/ccr3.2073](https://doi.org/10.1002/ccr3.2073)