Asian Journal of Surgery (2017) xx, 1–6

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-asianjournalsurgery.com

ORIGINAL ARTICLE

Clinical manifestations and *STK11* germline mutations in Taiwanese patients with Peutz–Jeghers syndrome

Jy-Ming Chiang [a,b,*,1], Tse-Ching Chen [b,c,1]

[a] *Division of Colon and Rectal Surgery, Department of Surgery, Chang Gung Memorial Hospital, Lin-Kou Medical Center, Tao-Yuan, Taiwan*
[b] *Chang Gung University, College of Medicine, Tao-Yuan, Taiwan*
[c] *Department of Pathology, Chang Gung Memorial Hospital, Lin-Kou Medical Center, Tao-Yuan, Taiwan*

Received 2 May 2017; received in revised form 14 July 2017; accepted 4 August 2017

KEYWORDS
Peutz–Jeghers syndrome;
STK11 gene;
Hamartomatous polyps

Summary *Backgrounds:* Clinical manifestations and molecular basis of Taiwanese patients with Peutz–Jeghers syndrome (PJS) were investigated to add the knowledge of phenotype and genotype of the disease.
Methods: Based on the Pathology Data Bank and the Colorectal Cancer Register, we collected their clinical data. The entire coding sequence of the *STK11* gene was amplified and analyzed by sequencing using the genomic DNA.
Results: Fifteen patients diagnosed with PJS from 11 unrelated families were collected until 2015. The median age at the onset of symptoms was 19 years with intussusception as the most frequent presenting symptom. Ten patients developing 11 cancers at various anatomical sites, including two cases of sinonasal cancer, two lung cancers, two breast cancers, two rectal cancers, two gynecological cancers and one small bowel cancer. Five of the deceased patients had died of cancers. The median age of diagnosis of first cancer in the probands was 32 years. Seventy patients (7 of 10) diagnosed before age of 40. Mutations found in eight families included five novel mutations (exon 6, c.843 ins G; exon 8, c.2065 delete A; exon 8, c.G923A, nonsense; exon 6, c.748dupA; and mTOR c.5107dupA) and three previously reported mutations. The other three PJS families without detectable *STK11* mutations did not develop malignancies so far.
Conclusion: This is the first comprehensive study of patients with Peutz–Jeghers syndrome in the Taiwanese. We have demonstrated that the phenotype of Peutz–Jeghers syndrome varies

Conflicts of interest: All the authors do not accept any financial or other interest that is relevant to the subject matter under consideration in this article.

* Corresponding author. Division of Colorectal Surgery, Chang Gung Memorial Hospital, No. 5, Fu-Hsing St., Kuei-Shan, Tao-Yuan 333, Taiwan. Fax: +886 03 3278355.
E-mail addresses: jmjiang1234@yahoo.com.tw, jmjiang@cgmh.org.tw (J.-M. Chiang), ctc323@cgmh.org.tw (T.-C. Chen).
[1] J-M Chiang and T-C Chen contributed equally to this study.

http://dx.doi.org/10.1016/j.asjsur.2017.08.002

Please cite this article in press as: Chiang J-M, Chen T-C, Clinical manifestations and *STK11* germline mutations in Taiwanese patients with Peutz–Jeghers syndrome, Asian Journal of Surgery (2017), http://dx.doi.org/10.1016/j.asjsur.2017.08.002

greatly among the patients. Patients with detectable STK11 mutations have very high risk of developing cancers.

1. Introduction

Peutz–Jeghers syndrome (PJS) is an inherited disorder associated with intestinal hamartomatous polyps. Patients with PJS significantly increased lifetime risk of intestinal and extra-intestinal malignancy. WHO diagnostic criteria for Peutz–Jeghers syndrome include two of the following three: three or more histopathologically proven hamartomatous polyps, mucocutaneous pigmentation and a positive family history.[1] Mucocutaneous pigmentations develop in infancy while polyps presented in adolescence and early adulthood. The number of hamartomatous polyps varies from few to hundreds, mostly located in the small bowel and colon but polyps may also be found extra-intestinally.[2,3]

Over 70% of patients with PJS have been detected with a pathogenic mutation in *STK11* gene (OMIM*602216 Serine/Threonine Protein Kinase 11). Acting as a tumor suppressor gene STK11 gene regulates cell metabolism, cell polarity, and cell proliferation.[4] Most of the cancers observed were associated with mutation located within the kinase domain, spanning exon 2 to exon 7, involved in substrate recognition and underwent phosphorylation on serine and threonine.[5]

With respect to the molecular characterization of PJS patients, there were similar cumulative overall and specific cancer risks between the mutation types (missense and truncating mutations).[6] Data from the genotype–phenotype correlation studies in PJS were inconsistent.[6–8] The type or site of STK11 mutation did not significantly affect the risk of malignancies.[8,9] Furthermore, age related cumulative risks of developing cancer were similar in patients with *STK11* mutations compared with those without detectable mutations.[6,7,9]

In this study, we try to investigate the clinical characteristics and molecular basis of in Taiwanese with PJS in order to add the knowledge of phenotype and genotype of the disease.

2. Patients and methods

The patients in this study were identified from records in the pathological department and colorectal cancer registry in Chang Gung Memorial Hospital. Subjects enrolled from pathological department were individuals with a clinical diagnosis of hamartomatous polyps fulfilled with WHO criteria of PJ syndrome were included. Criteria for exclusion were presence of solitary hamartomatous polyps or presence of only mucocutaneous pigmentation in isolated cases. When $\geq$2 individuals were affected in the same family, they were considered as familial cases. In families with 1 individual with PJS, those who had only gastrointestinal hamartomatous polyposis were also counted as affected members. Pathology reports and medical/surgical records are obtained to confirm cancer diagnosis. All information was collected before the end of 2015.

Clinical data were collected by reviewing patients' medical records. Characteristic features collected including age at first symptoms, presence of pigmentation, localization of the polyps, histology of the polyp biopsy, presence of cancer and its histology type, organ involved, age at cancer diagnosis, and the follow-up period.

The study was performed with ethical committee approval from the Chang Gung Memorial Hospital. After informed consent, blood or paraffin embedded tissue samples were obtained from the patients' surgical specimens. Normal tissue and tumor DNA were collected from paraffin block. DNA isolation from blood or tissue sample was performed by using the Gentra kit (Amersham, Buckinghamshire, UK) according to the manufacturer's recommendations. Polymerase chain reaction (PCR) products were resolved by electrophoresis and visualized by autoradiography.

Genetic diagnosis of PJ syndrome was established by identifying mutations of the *STK11* gene. Mutation analysis was performed using single-strand conformational polymorphism (SSCP) to screen the 9 *STK11* exons and flanking intronic sequences in genomic DNA extracted from blood leukocytes or tissue samples. For patients who were dead, we can get paraffin blocks from our tumor bank in the Department of Pathology. DNA was extracted after microdissection of the paraffin blocks. For patients who were still alive, in addition to paraffin block tissues we also obtained DNA from peripheral blood for some of them. PCR and gel conditions have been previously reported.[8] PCR products that revealed aberrant bands by SSCP analysis were subjected to DNA sequencing for confirmation. Recently, next generation sequencer was used as well. Next-generation sequencing libraries were prepared using the AmpliSeq Library Kit 2.0 (Thermo Fisher Scientific). Sequencing was performed on an Ion Torrent sequencer (Thermo Fisher Scientific). Initial data analysis was performed using the Ion Torrent Suite Software (Thermo Fisher Scientific, open source, GPL, https://github.com/iontorrent/). *STK11* sequence analyses of genomic DNA from patients were performed using our own design gene panel including *STK11* and some common colorectal polyposis related genes. The panel thus includes STK11 (*LKB1*), *MO25*, *mTOR*, *MYH11*, *EGFR*, *P1K3CA*, *AKT*, *KRAS*, *PTEN*, *APC*, *TP53*, *MARK1*, *MARK2*, *MARK3*, *MARK4*. Mutations in the *STK11* gene identified in the PJS families were coded according to the published sequence of the

Please cite this article in press as: Chiang J-M, Chen T-C, Clinical manifestations and *STK11* germline mutations in Taiwanese patients with Peutz–Jeghers syndrome, Asian Journal of Surgery (2017), http://dx.doi.org/10.1016/j.asjsur.2017.08.002