RESEARCH NOTE

Two variants in *STK11* gene in Chinese patients with Peutz–Jeghers syndrome

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[Liu D., Guo H., Xu X., Yu Y. and Bai Y. 2012 Two variants in *STK11* gene in Chinese patients with Peutz–Jeghers syndrome. *J. Genet.* **91**, 205–208]

Introduction

Peutz–Jeghers syndrome (PJS) is an autosomal dominant disease manifested as multiple hamartomatous polyps of gastrointestinal tract, mucocutaneous pigmentation and increased risk of cancers. The typical clinical symptoms of PJS are abdominal pain, intestinal obstruction, volvulus and anaemia. The causative gene for PJS was localized to chromosome 19p13.3, further studies identified variants of *STK11* gene were found in most PJS patients (Beggs *et al*. 2010). The human *STK11* gene that encodes serine–threonine kinase is thought to be a tumour suppressor gene and has wide biological effects such as regulating cellular proliferation by G1 cell-cycle arrest (Tiainen *et al*. 1999), p53 mediated apoptosis (Karuman *et al*. 2001), cell polarity (Morton *et al*. 1992), regulating AMP activated protein kinase (AMPK) and Wnt signalling pathway (Lin-Marq *et al*. 2005). In this study, we reported two variants of *STK11* (p.Thr363Ile and p.Phe354Leu), which were related with PJS in two Chinese families.

Materials and methods

Patients

Two probands from two unrelated families were recruited for this study. One had a positive family history and the other was sporadic. Clinical diagnosis for PJS was based on the presence of any one of the following clinical findings: (i) two or more histologically confirmed PJ polyps in the gastrointestinal tract; (ii) any number of PJ polyps detected with a positive family history of PJS; (iii) characteristic mucocutaneous pigmentation with a positive family history of PJS; and (iv) any number of PJ polyps together with characteristic mucocutaneous pigmentation (Beggs *et al*. 2010). Pedigrees of the two families are shown in figure 1.

Sample collecting and variant detection

After informed consent was obtained from the family members, venous blood samples were collected from the two probands and their family members. Meanwhile, 150 blood samples from unrelated healthy persons were selected as controls. The study was performed with the approval of the Ethics Committee of Third Military Medical University (Chongqing, China). Extraction of genomic DNA was performed using Wizard Genomic DNA Purification kit (Promega, Madison, USA) according to the protocol.

The software Primer Premier 5.0 was used to design the primers to amplify the whole exons and the exon–intron boundaries of the *STK11* gene (conditions and the primer pairs for PCR are available on request). PCR products were purified with purification kit (Tiangen, Beijing, China). Purified PCR products were sequenced by ABI 3130 genetic analyser (Foster City, USA).

Functional significance prediction

Three online softwares including PolyPhen 2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org/) and Swiss-Model (http://swissmodel.expasy.org/) were used to predict the functional significances of the variants.

Results

Clinical features

Family 1: This family showed inheritance of PJS as an autosomal dominant. The medical history of the proband revealed

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Keywords. Peutz–Jeghers syndrome; serine–threonine kinase 11; family; mutation.

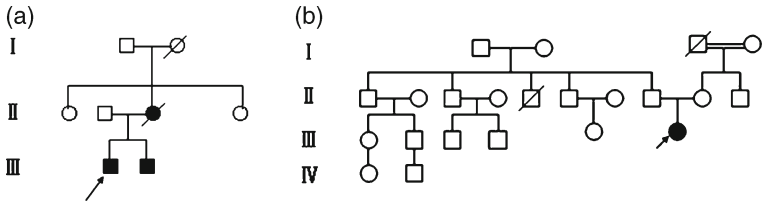

Figure 1. Pedigrees of the two unrelated families with PJS. (a) And (b) indicate the pedigrees of family 1 and family 2, respectively. The probands are indicated by the arrow.

that the mucocutaneous pigmentation appeared at the age of 3 and developed abdominal pain and bloody stool at the age of 12 years. Endoscopic examination showed the whole colorectal multiple polyps. Some large polyps were removed by polypectomy under enteroscopy. The polyps removed were histological confirmed as hamartomatous polyps. The mucocutaneous pigmentation was obvious especially around lips (figure 2a). The mother of the proband died of malignant lymphoma at the age of 39, who had mucocutaneous pigmentation at 13 years. The younger brother of the proband was a five-year-old boy, who had some similar clinical features such as mucocutaneous pigmentation and rectal multiple polyps.

Family 2: The proband, a 16-year-old girl, who had been operated six years ago because of multiple polyps in stomach and duodenum. The polyps removed were histological confirmed as hamartomatous polyps. Physical examination found that the mucocutaneous pigmentation was around lips, digits and perineal region, especially in digits (figure 2b). The parents had no mucocutaneous pigmentation and gastrointestinal polyps.

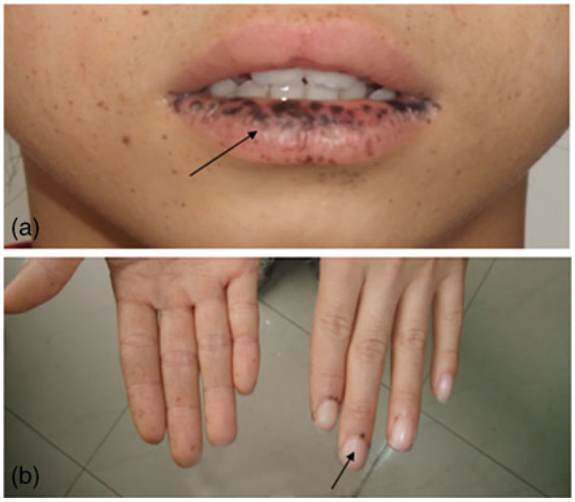

Figure 2. Clinical features of two probands. (a) Indicates perioral melanin pigmentation spots of the lips in the proband of family 1; (b) indicates melanin pigmentation spots of nails in the proband of family 2. The melanin pigmentation is indicated by the arrow.

Variant detection

Sequencing analysis showed that two variants, c.1088 C>T and c.1062 C>G, at exon 8 of *STK11* gene were found in both probands respectively, and in family 1 the c.1088 C>T variant was also identified in the proband's younger brother. No other sequence changes were observed. No variant of *STK11* gene was detected in two family healthy members and 150 healthy controls (shown in figure 3).

The two variants resulted in substitution of threonine to isoleucine at the 363 codon (p.Thr363Ile) and from phenylalanine to leucine at the 354 codon (p.Phe354Leu) of serine/threonine-protein kinase11.

Functional significance prediction

The p.Thr363Ile and p.Phe354Leu substitutions were rated as benign by PolyPhen and SIFT softwares. In addition, the protein structures of the two variants were not changed compared to wild STK11 protein by the prediction of Swiss-Model software.

Discussion

Roles of STK11 in PJS pathogenesis

STK11 protein is mainly comprised of three major domains: N-terminal noncatalytic domain, the catalytic kinase domain and the carboxyterminal noncatalytic regulatory domain. The catalytic kinase domain is encoded by amino acids 49–309, which could form a complex with STRAD and MO25 to maintain kinase activation (Boudeau *et al*. 2003), and the C-terminal noncatalytic region is encoded by exon 8/9. Most of variants in PJS patients are located in the region for catalytic kinase domain and result in absence of kinase activity, thus disrupting the functions of STK11.

The variant p.Phe354Leu found in family 2 was reported in a previous study (Amos *et al*. 2004), which was located at the C-terminal region of STK11 protein (Yoo *et al*. 2002). The functional significances of p.Phe354Leu were demonstrated in Forcet's study (Forcet *et al*. 2005). The results provided evidence that the variant p.Phe354Leu neither disrupted STK11 kinase activity nor interfered with STK11-induced growth arrest. However, it could lessen STK11-mediated activation of the AMP-activated protein kinase

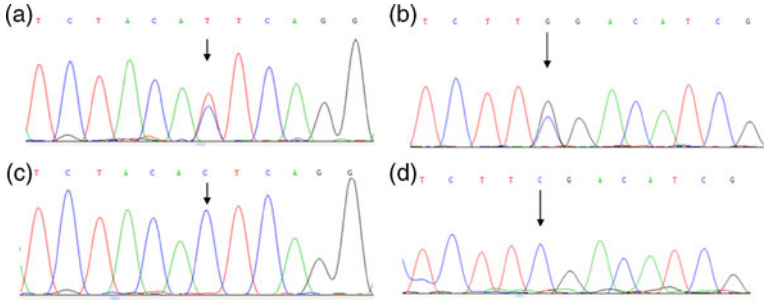

Figure 3. Sequencing results of the *STK11* gene. (a) The variant (c.1088 C>T) at exon 8 of *STK11* in the proband of family 1. (b) The variant (c.1062 C>G) at exon 8 of *STK11* in the proband of family 2. (c) And (d) indicate the corresponding normal sequence of*STK11* in healthy control. The localizations of the variants are indicated by the arrows.

(AMPK) and impair downstream signalling. Further, this variant compromised STK11 protein ability to establish and maintain polarity of both intestinal epithelial cells and migrating astrocytes.

In family 1, we found a novel variant at exon 8 of *STK11* gene, which resulted in an amino acid change from Thr363 to Ile (p.Thr363Ile). Thr363 is a phosphorylated site located in C-terminal region, which is highly conversed in mammalian. Sapkota *et al*. (2002) had reported that Thr366 was phosphorylated by both the ataxia telangiectasia mutated kinase and the DNA-dependent protein kinase *in vivo*. Variant from Thr366 to Ala or Asp did not affect STK11 kinase activity *in vitro* or STK11 localization *in vivo* (Sapkota *et al*. 2002). However, there has not been any study on its functional effects. Based on the amino acid residues of STK11 protein, Thr363 lies in nine residues away from Phe354. So the variant p.Thr363Ile might also influence STK11 activity by interrupting phosphorylation and posttranslational events, which needs to be determined in further studies.

Cancer risk

Several reports have demonstrated that PJS patients had higher risk of developing cancers. Hearle *et al*. (2006) evaluated the incidence of cancers in 419 individuals with PJS, 96 cancers were found and the risk for developing cancer at ages 20, 30, 40, 50, 60, and 70 years was 2%, 5%, 17%, 31%, 60% and 85%, respectively (Hearle *et al*. 2006). The study also concluded that the most common cancers appeared in PJS patients were gastrointestinal in origin, gastroesophageal, small bowel, colorectal, and pancreatic cancer, and the major risk of extraintestinal malignancy in female patients was breast cancer.

The substantial cancer risk associated with PJS support the need for surveillance in PJS patients for early detection of tumours. Several clinical centres have proposed guidelines for screening patients with PJS. Most centres advocate upper and lower endoscopy and breast examination and some advocate surveillance for pancreatic and gynaecologic malignancies. Because the relationship between the type and site of *STK11* gene variant and cancer risk is not certain, surveillance strategies should be applied in all PJS patients.

In conclusion, two variants (p.Thr363Ile and p.Phe354Leu) were found at *STK11* in two unrelated Chinese PJS families, which could be the pathogenic cause for PJS. The results enlarged the spectrum of *STK11* variants in PJS patients.

Acknowledgements

We thank the patients and their clinicians for participating in this study.

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Received 29 August 2011, in final revised form 29 December 2011; accepted 20 February 2012
Published on the Web: 4 June 2012