[2] Wong GL, Tse YK, Wong VW, Yip TC, Tsoi KK, Chan H. Long-term safety of oral nucleos(t)ide analogs for patients with chronic hepatitis B: a cohort study of 53,500 subjects. Hepatology 2015;62:684–93.
[3] Lampertico P, Chan HL, Janssen HL, Strasser SI, Schindler R, Berg T. Review article: long-term safety of nucleoside and nucleotide analogues in HBV-monoinfected patients. Aliment Pharmacol Ther 2016;44:16–34.
[4] Gupta SK. Tenofovir-associated Fanconi syndrome: review of the FDA adverse event reporting system. AIDS Patient Care STDS 2008;22:99–103.
[5] Suh YS, Chun DI, Choi SW, Lee HW, Nho JH, Kwon SH, et al. Pathologic femoral fracture due to tenofovir-induced Fanconi syndrome in patient with chronic hepatitis B. Medicine (Baltimore) 2017;96:46.
[6] Grossi G, Loglio A, Facchetti F, Borghi M, Soffredini R, Galmozzi E, et al. Tenofovir alafenamide as a rescue therapy in a patient with HBV-cirrhosis with a history of Fanconi syndrome and multidrug resistance. J Hepatol 2018;68:195–8.
[7] Kim D, Lee J, Kim DH, Kang K, Suh SJ, Jung YK, et al. A case of tenofovir-associated fanconi syndrome in patient with chronic hepatitis B. Korean J Gastroenterol 2016;68:317–20, http://dx.doi.org/10.4166/kjg.2016.68.6.317.
[8] López Centeno B, Collado Borrell R, Pérez Encinas M, Gutiérrez García ML, Sanmartin Fenollera P. Comparison of the effectiveness and renal safety of tenofovir versus entecavir in patients with chronic hepatitis B. Farm Hosp 2016;40:279–86.
[9] Pascale R, Guardigni V, Badia L, Volpato F, Viale P, Verucchi G. Early onset of tenofovir-related fanconi syndrome in a child with acute hepatitis B: a case report and systematic review of literature. Case Rep Hepatol 2017:3921027.
[10] EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus Infection. J Hepatol 2017;67:370–98.

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Early genetic testing of *STK11* is important for management and genetic counseling for Peutz–Jeghers syndrome

To the Editor,

Peutz–Jeghers syndrome (PJS) is a rare autosomal dominant inherited polyposis disorder (OMIM #175200), with an estimated incidence varying from 1/8300 to 1/280000 live births [1]. It is characterized by multiple gastrointestinal (GI) hamartomatous polyps, and mucocutaneous pigmentation, particularly on the vermilion border of lips. PJS patients have an increased lifetime risk of GI cancers and a wide spectrum of extra-GI malignancies [2,3]. More than 90% of PJS patients carry loss-of-function mutations at *serine-threonine kinase 11*(*STK11*, also named as *LKB1*, OMIM #602216), which is an important tumor suppressor gene controlling cell growth, division and apoptosis [4]. About 25% of PJS patients have de novo mutations of this gene [5]. PJS shows some clinical heterogeneity, such that pigmented lesions and gastrointestinal symptoms may not always be demonstrated in an affected individual [5]. Affected individuals, particularly those without family history of the condition, often pay insufficient attention if symptoms are not obvious, until they have to receive surgical treatment or develop cancer. Early diagnosis of this disease can be quite helpful for treatment and cancer surveillance and to improve life quality of PJS patients. Genetic testing for *STK11* gene is undoubtedly a promising and effective way.

Here, we report two unrelated Chinese PJS families, and identify a novel and probably *de novo* "pathogenic" mutation (c.1004_1005insT [p.Met335IlefsTer25]). The two families were unrelated and from Hubei province of China.

Family 1

A 12-year-old boy was referred for diagnosis for his growing mucocutaneous pigmentation. His father had similar symptoms at youth, but he did not pay attention until he suffered advanced colorectal cancer and then died of it at the age of 37 years. The boy had characteristic pigmentation on his lips, buccal mucosa and two hands. Pigmentation initially appeared on his lower lip at 6 months after birth, then increased with age and appeared on his fingers in recent months. Although the boy reported no GI symptoms (such as abdominal pain, diarrhea, and hematochezia), endoscopic examination found some polyps in the stomach and colon. The polyps were confirmed to be hamartomatous by pathological examination after endoscopic polypectomy and the boy was diagnosed with PJS. His father had mucocutaneous pigmentation and polyps, and died at the age of 37 due to colorectal cancer. His mother showed no features of PJS.

Family 2

A 30-year-old man was referred for further treatment of GI polys and for relevant genetic counseling. He had characteristic mucocutaneous freckling and reported years of intermittent abdominal pain. In 2005, he was diagnosed with polyposis and intestinal obstruction, and underwent surgical resection due to partial intestinal necrosis. Then, he received several surgical treatments because of multiple polyps on esophagus, stomach, small bowel and colon. The pathological examination revealed hamartomatous polyps. His mother, who had a similar history of intestinal obstruction at age 10 years, was also diagnosed with PJS based on pigmented lesions and GI hamartomatous polyps. Moreover, the patient and his affected mother had pigmentation initially at the age of 3 years. His 3-year-old daughter who presented two freckles in her left hand, and he wanted to know the risk of PJS for his daughter. No other family members demonstrated any PJS features.

Based on written informed consents, we collected blood samples from the probands of both families and their available family members. Genomic DNA was extracted using QIAamp DNA blood mini kit (Qiagen GmbH, Germany), and then subjected to polymerase chain reactions (PCR) to amplify the entire coding regions and splice boundaries of *STK11* gene (NP_000446.1, NM_000455.4, GRCh38.p7). The purified PCR products were analyzed by bidirectional sequencing using the ABI 3500 DNA sequencer (Applied Biosystems)

In family 1, a heterozygous 9-bp deletion, c.907_915delATCCGGCAG (p.Ile303_Gln305del), was detected in the boy (Fig. 1a and 1b, II: 1). The germline deletion was not detected in his unaffected mother (Fig. 1a and 1b, I: 2) and was speculated to be inherited from his affected father. The deletion was in exon 7 of *STK11* and could lead to loss of three amino acids in the kinase domain (Fig. 1e). This mutation has not been recorded in multiple population databases including Exome Aggregation Consortium (ExAC), 1000 Genomes Project, genomeAD and dbSNP. Integrative databases of MutationTaster and PROVEAN predicted that it was deleterious based on evidence including evolutionary conservation, protein features and structure. This mutation was also detected in a 14-year-old Thai girl in a previous report [6]. Taken together, c.907_915delATCCGGCAG (p.Ile303_Gln305del) is considered as a "likely pathogenic" mutation in *STK11* causing

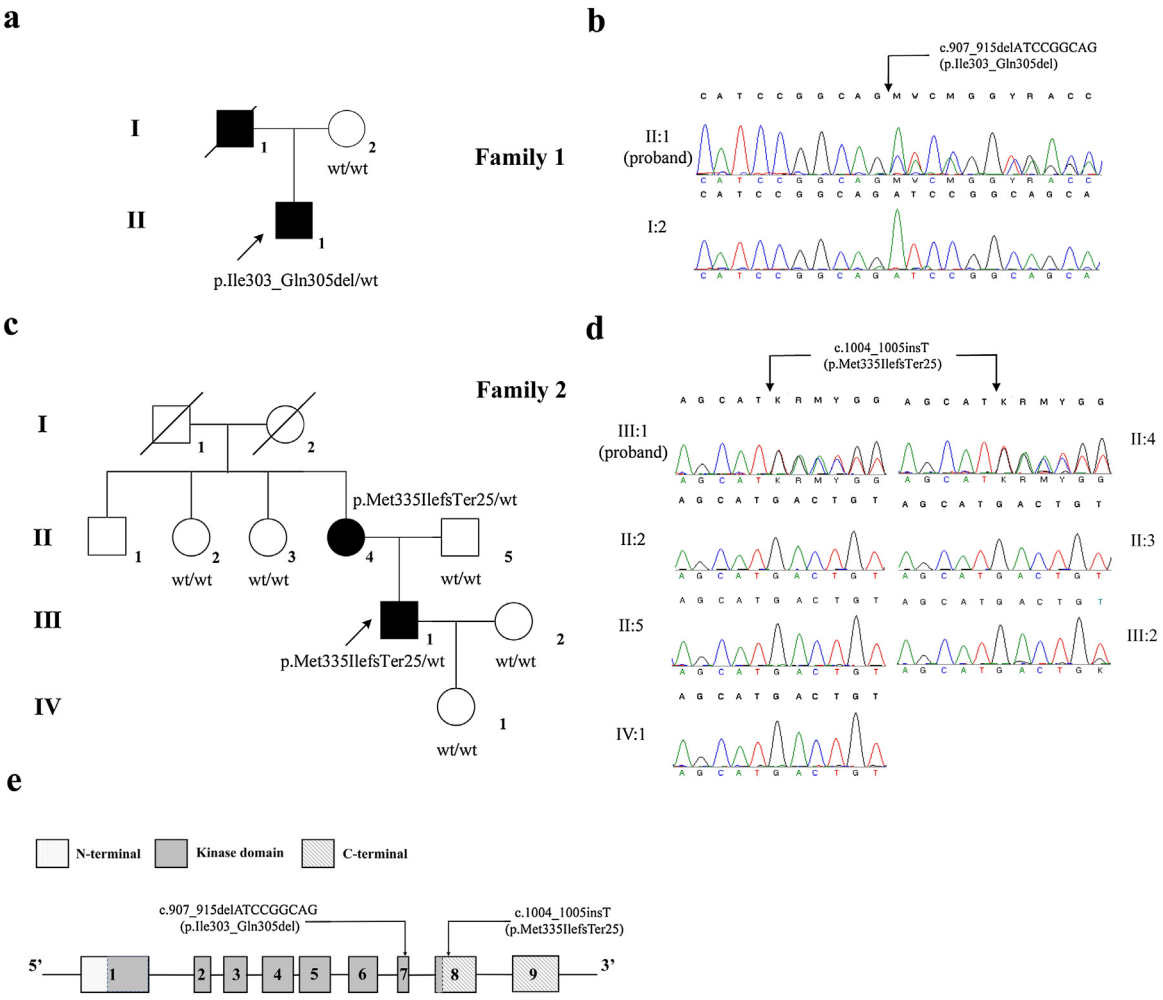

Fig. 1. Pedigree of the PJS family 1 (a) and Sanger sequencing results (b). The proband's father died three years ago and was unavailable for mutation analysis. Pedigree of the PJS family 2 (c) and Sanger sequencing results (d). Three unaffected family members failed to receive genetic testing based on the following reasons: the proband's maternal grandfather and grandmother died several years ago, and his maternal uncle is more than 70 years and lives abroad. (e) The gene structure of *STK11*. c.907_915delATCCGGCAG (p.Ile303_Gln305del) is located in exon 7 within the kinase domain. c.1004_1005insT (p.Met335IlefsTer25) is located in exon 8 within the C-terminal regulatory region. wt, wild type. Squares indicate males and circles indicate females. Affected individuals are marked by solid symbols and unaffected individuals are marked by open symbols. The proband is denoted by an arrow.

Table 1
Classification of detected genetic variants according to ACMG guidelines.

	c.907_915delATCCGGCAG (p.Ile303_Gln305del)	c.1004_1005insT (p.Met335IlefsTer25)
Population data[a]	Absent in multiple normal population databases, including ExAC, 1000GP, genomeAD and dbSNP (PM2)	Absent in multiple general population databases, including ExAC, 1000GP, genomeAD and dbSNP (PM2)
Deletions/Insertions	Changed protein length because of in-frame deletions in a non-repeat region (PM4)	Located in exon 8 (but not at the 3′-most 50 bp of the penultimate exon) and predicted to cause the premature termination codon for NMD (PVS1)
De novo data	NA	Assumed de novo without paternal and maternal confirmation (PM6)
Segregation data	NA	Co-segregation with PJS in multiple affected family members (PP1)
Computational evidences[b]	Multiple computational evidences (MutationTaster and PROVEAN) support a deleterious effect on *STK* gene product including evolutionary conservation, protein features and structure (PP3)	Disease-causing predicated by MutationTaster
Phenotype support	Carriers have typical PJS features, including mucocutaneous pigmentation (particularly on vermillion border of the lips) and hamartomatous polyps (PP4)	Carriers have typical PJS features, including mucocutaneous pigmentation (particularly on vermillion border of the lips) and hamartomatous polyps (PP4)
Conclusion	Likely pathogenic (2 PM and 2 PP)	Pathogenic (1 PVS1 and 2 PM)

Abbreviation: ACMG, American College of Medical Genetics and Genomics; ExAC, Exome Aggregation Consortium; 1000GP, 1000 Genomes Project; NMD, nonsense-mediated decay; PVS, pathogenic very strong; PM, pathogenic moderate; PP, pathogenic supporting; NA, not available.

[a] ExAC, http://exac.broadinstitute.org/); 1000GP, http://www.internationalgenome.org/1000-genomes-browsers; genomeAD, https://gnomad.broadinstitute.org/; dbSNP, https://www.ncbi.nlm.nih.gov/snp/.

[b] MutationTaster, http://www.mutationtaster.org/; PROVEAN, http://provean.jcvi.org/index.php.

PJS according to the guidelines of American College of Medical Genetics and Genomics (ACMG) (Table 1).

In family 2, co-segregation analysis demonstrated that the proband (III: 1 in Fig. 1c and 1d) inherited a heterozygous insertion, c.1004_1005insT (p.Met335IlefsTer25), from his affected mother (Fig. 1c and 1d, II: 4), but did not transfer it to his daughter (Fig. 1c and 1d, IV: 1). It was a translational frameshift mutation in exon 8 of *STK11*, resulting in a premature stop codon and loss of the C- terminal regulatory domain (CRD) (Fig. 1e). Mutations within this CRD could attenuate the activity of AMP-activated protein kinase, impair downstream signaling, and affect the polarity of cells [7]. Based on available evidences, we considered that c.1004_1005insT (p.Met335IlefsTer25) was a novel and probably *de novo* "pathogenic" mutation of *STK11* (Table 1).

In general, PJS can be easily diagnosed based on characteristic features (mucocutaneous pigmentation and GI hamartomas). However, typical manifestations may not always appear in an affected individual and/or draw sufficient attention, especially in those without positive family history. In this case, the individual is often prone to develop serious outcomes, leading to inevitable open surgery or even cancer [8]. In family 1, the affected boy's father neglected his fading pigmentation and inconspicuous GI symptoms until he was diagnosed with advanced colorectal cancer. Fortunately, the tragedy increased awareness and allowed timely diagnosis and treatment for the boy. Our identified likely pathogenic mutation (c.907_915delATCCGGCAG [p.Ile303_Gln305del]) provided molecular diagnostic evidence for PJS, and it could be used for early diagnosis of individuals whose family has a history of the condition.

Usually, individuals with positive family history desire to know the risk of PJS on themselves and their offspring. Genetic testing of *STK11* gene is an effective way to identify potential PJS patients ahead of onset of symptoms, and provide predictive diagnosis, further management and genetic counseling for affected pedigrees. In the case of family 2, we identified that the proband inherited a novel heterozygous pathogenic mutation (c.1004_1005insT [p.Met335IlefsTer25]) from his affected mother, but fortunately did not transmit it to his daughter, greatly relieving their stress and anxiety. If they have future pregnancies, prenatal genetic testing or preimplantation genetic diagnosis (PGD) could help them avoid an affected baby based on the identified pathogenic mutation. In fact, prenatal diagnosis of PJS by genetic testing of *STK11* gene has been successfully performed in India [9] and China [10].

In summary, we report clinical and molecular characteristics of two unrelated PJS families with different outcomes, and identified a novel and probably *de novo* "pathogenic" mutation (c.1004_1005insT [p.Met335IlefsTer25]) of *STK11* gene. Our work not only highlights the broad pathogenic mutation spectrum of PJS, but also emphasizes the importance of genetic testing and counseling in high-risk individuals.

Competing interest

None declared.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China), and was performed in accordance with the principles of Declaration of Helsinki. Written informed consents for clinical information, blood samples and paper publication were obtained from all included subjects or guardians.

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References

[1] Lindor NM, McMaster ML, Lindor CJ, Greene M. Concise handbook of familial cancer susceptibility syndromes. Second edition; 2008.
[2] Giardiello FM, Brensinger JD, Tersmette AC, Goodman SN, Petersen GM, Booker SV, et al. Very high risk of cancer in familial Peutz–Jeghers syndrome. Gastroenterology 2000;119:1447–53.
[3] Chen HY, Jin XW, Li BR, Zhu M, Li J, Mao GP, et al. Cancer risk in patients with Peutz–Jeghers syndrome: a retrospective cohort study of 336 cases. Tumour Biol 2017;39, 1010428317705131.
[4] Lizcano JM, Goransson O, Toth R, Deak M, Morrice NA, Boudeau J, et al. LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. EMBO J 2004;23:833–43.
[5] Achatz MI, Porter CC, Brugières L, Druker H, Frebourg T, Foulkes WD, et al. Cancer screening recommendations and clinical management of inherited gastrointestinal cancer syndromes in childhood. Clin Cancer Res 2017;23:e107–14.
[6] Ausavarat S, Leoyklang P, Vejchapipat P, Chongsrisawat V, Suphapeetiporn K, Shotelersuk V. Novel mutations in the STK11 gene in Thai patients with Peutz–Jeghers syndrome. World J Gastroenterol 2009;15:5364–7.
[7] Forcet C, Etienne-Manneville S, Gaude H, Fournier L, Debilly S, Salmi M, et al. Functional analysis of Peutz–Jeghers mutations reveals that the LKB1 C-terminal region exerts a crucial role in regulating both the AMPK pathway and the cell polarity. Hum Mol Genet 2005;14:1283–92.
[8] van Lier MG, Mathus-Vliegen EM, Wagner A, van Leerdam ME, Kuipers EJ. High cumulative risk of intussusception in patients with Peutz–Jeghers syndrome: time to update surveillance guidelines? Am J Gastroenterol 2011;106:940–5.
[9] Thakur N, Reddy DN, Rao GV, Mohankrishna P, Singh L, Chandak GR. A novel mutation in STK11 gene is associated with Peutz–Jeghers syndrome in Indian patients. BMC Med Genet 2006;7:73.
[10] Wang Z, Liu S, Liu S, Wang Y, Chen J, Wu B. Prenatal diagnosis in a hereditary Peutz–Jeghers syndrome family with high cancer risk. BMC Med Genet 2018;19:66.

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Noninvasive prediction model for diagnosing gastrointestinal stromal tumors using contrast-enhanced harmonic endoscopic ultrasound: Methodological issues

Dear Editor,

We read with great interest the recent paper by Cho et al., published in the *Digestive and Liver Disease journal*, entitled "*Noninvasive prediction model for diagnosing gastrointestinal stromal*