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.

Acknowledgments

The authors thank patients and their family members for participation.

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.

Na Shen
Delei Li
Yaowu Zhu
Department of Laboratory Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Huaping Xie*
Department of Gastroenterology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Yanjun Lu**
Department of Laboratory Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

* Corresponding author.

** Corresponding author.
E-mail addresses: rainerxie@126.com (H. Xie), junyanlu_2000@163.com (Y. Lu).

28 May 2019

https://doi.org/10.1016/j.dld.2019.06.003

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*

tumors using contrast-enhanced harmonic endoscopic ultrasound" [1].

They aimed at creating a noninvasive prediction model for diagnosing gastrointestinal stromal tumors (GISTs) using contrast-enhanced harmonic endoscopic ultrasound (CEH-EUS). We would like to mention some methodological issues to avoid misleading interpretation of the results. Firstly, the manner in which the authors recruited patients to the study provokes controversy. Actually, they enrolled convenient participants while no appropriate sample size calculation and selection strategy were determined in the study design. Therefore, the sample size could be subjected to the selection bias. Secondly, the authors introduced factors such as the positive vascularity (Odds Ratio (OR) = 27.765, 95% Confidence Interval (CI) = 5.336–144.458) and low long-to-short ratio (LSR) (OR = 18.940, 95%CI = 3.623–99.007) as the independent predictors of GIST. On the contrary, a big estimated ORs with wide CIs does not always mean a strong predictor, it could be due to the sparse data problem which leads to the biased estimation of parameters. It happens when the observations in the combination of exposure and outcome strata are rare. The authors could use appropriate statistical methods such as Firth penalization and Data Augmentation to reduce sparse data bias [2–4].

Finally, internal and external validities are essential parts of a prediction study to confirm the validity of the obtained results, while this study ignored these parts. There are several methods to investigate internal validity such as split-sample validation, cross-validation and bootstrapping. Regarding the small sample size of this study, we recommend bootstrapping approach [5]. External validity checking is also essential to support the general applicability of the prediction model [6]. We suggest considering the mentioned methodological issues to obtain reliable results.

Acknowledgment

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] Cho IR, Park JC, Roh YH, Choi SI, Lee JE, Kim EH, et al. Noninvasive prediction model for diagnosing gastrointestinal stromal tumors using contrast-enhanced harmonic endoscopic ultrasound. Digestive and Liver Disease.
[2] Firth D. Bias reduction of maximum likelihood estimates. Biometrika 1993;80(1):27–38.
[3] Firth D. Recent developments in quasi-likelihood methods. Bull Inst Stat Inst 1993;55:341–58.
[4] Heinze G, Schemper M. A solution to the problem of separation in logistic regression. Stat Med 2002;21(16):2409–19.
[5] Steyerberg E. Clinical prediction models: a practical approach to development, validation and updating. Springer Science & Business Media; 2008.
[6] Justice AC, Covinsky KE, Berlin JA. Assessing the generalizability of prognostic information. Ann Intern Med 1999;130(6):515–24.

Razieh Bidhendi Yarandi [a,b]
[a] *Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Iran*
[b] *Reproductive Endocrinology Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Iran*

Mohammad Hossein Panahi [*]
[c] *Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Iran*

[*] Corresponding author.
E-mail address:
mohammadpanahi55@yahoo.com (M.H. Panahi)

31 March 2019

https://doi.org/10.1016/j.dld.2019.04.017