

DATA REPORT

Open Access

Germline deletion of chromosome 2p16-21 associated with Lynch syndrome

Soichiro Natsume¹, Tatsuro Yamaguchi^{1,2}, Hidetaka Eguchi³, Yasushi Okazaki³, Shin-ichiro Horiguchi⁴ and Hideyuki Ishida⁵

Abstract

We identified a Japanese patient with Lynch syndrome with a novel large germline deletion of chromosome 2p16-21, including the *EPCAM*, *MSH2*, and *KCNK12* genes. The proband was a 46-year-old man with ascending colon cancer. The clinical significance of germline *KCNK12* gene deletion, which encodes one of the subfamilies of two-pore-domain potassium channels, is still unknown.

Lynch syndrome (LS) is an autosomal dominant inherited disorder caused by a germline pathogenic variant in mismatch repair (MMR) genes, including the *MLH1*, *MSH2*, *MSH6*, and *PMS2* genes. Inactivation of the MMR gene, which is caused by both germline and/or somatic variants, leads to an increased frequency of replication errors in repeated sequences in the coding regions of cancer-related genes, resulting in the development of LS-associated tumors¹. Therefore, LS is characterized by the development of various cancers at a young age, including colorectal cancer, endometrial cancer, small bowel cancer, and urinary tract cancer.

The *EPCAM* gene, which is located 17 kb upstream of *MSH2*, is not an MMR gene. However, germline deletion of the 3' end of the *EPCAM* gene causes LS because *EPCAM* deletion leads to *MSH2* gene silencing by promoter hypermethylation and *MSH2* inactivation². The proportion of *EPCAM* gene deletions in LS is estimated to be 1.0–3.0%^{3,4}, and to date, only a few families have been reported in Japan⁵.

Here, we identified a Japanese patient with LS with a novel large germline deletion of chromosome 2p16-21,

including the *EPCAM*, *MSH2*, *KCNK12* genes. This novel finding provides evidence of germline deletion in this region and raises questions regarding the deletion of the *KCNK12* gene.

The proband was a 46-year-old man who was referred to our hospital with abdominal pain. A colonoscopy demonstrated an ascending colonic tumor, and biopsy revealed adenocarcinoma in the ascending colon. However, computed tomography showed no evidence of lymph node metastasis, distant metastasis, or other organ diseases. In addition, his mother had been treated for colon cancer, glioblastoma, gastric cancer, and endometrial cancer, and she died from glioblastoma at the age of 67 years old (Fig. 1).

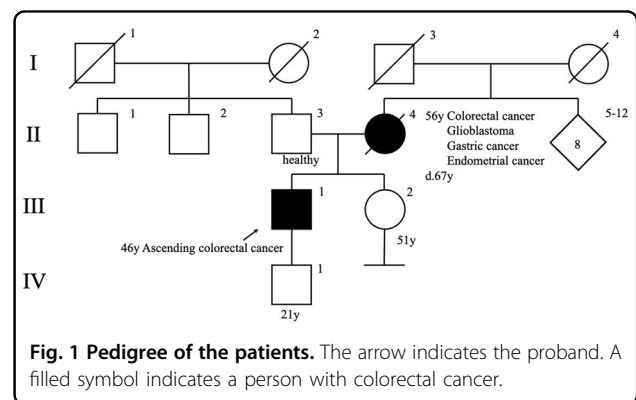


Fig. 1 Pedigree of the patients. The arrow indicates the proband. A filled symbol indicates a person with colorectal cancer.

Correspondence: Tatsuro Yamaguchi (tatsuro@yamaguchi.email.ne.jp)

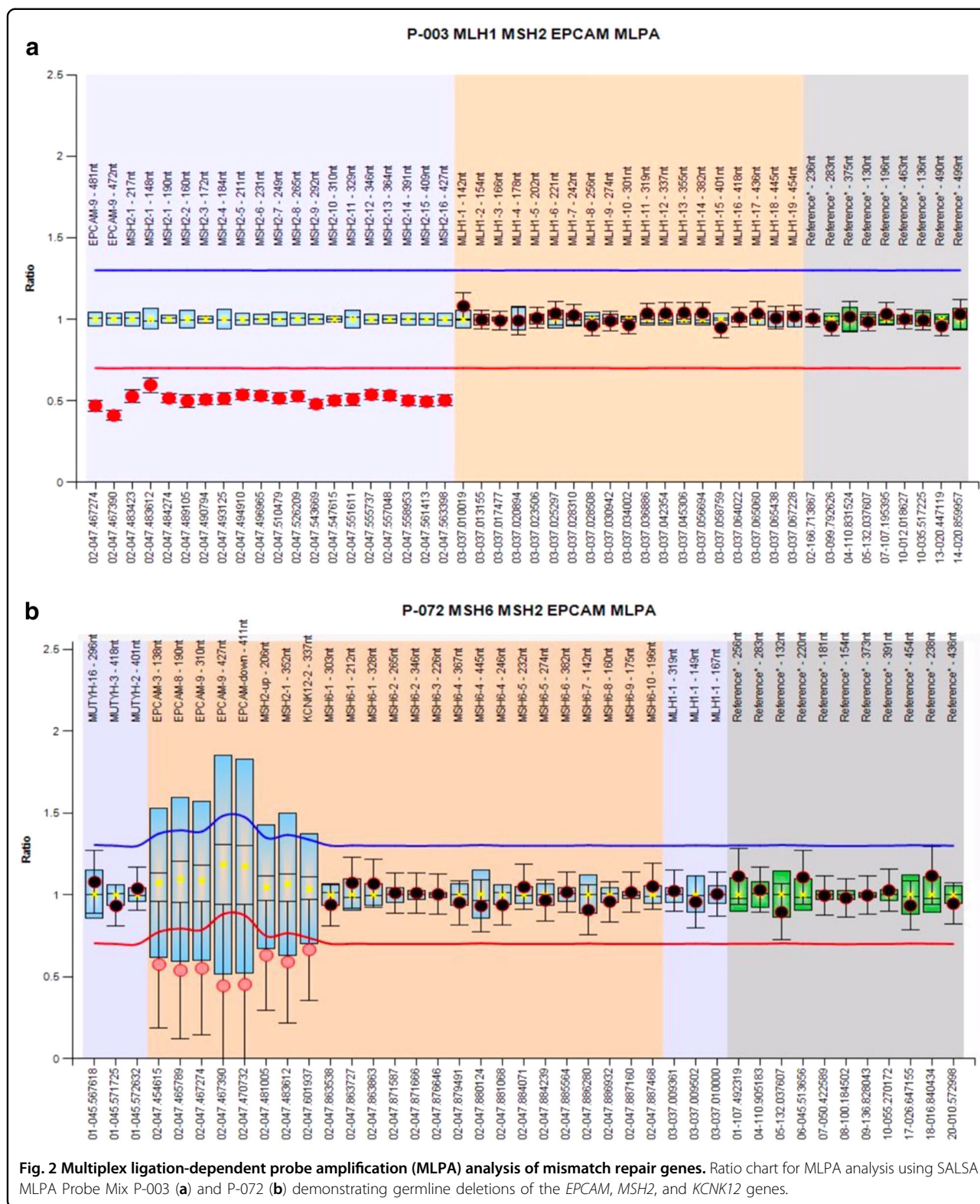
¹Department of Surgery, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan

²Department of Clinical Genetics, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan

Full list of author information is available at the end of the article

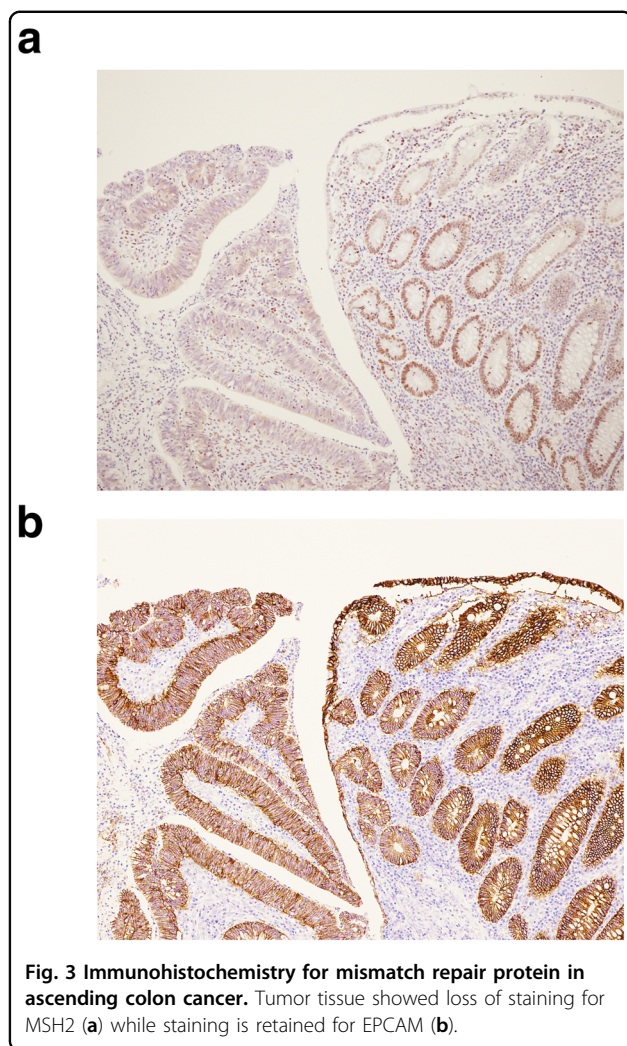
© The Author(s) 2021

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.



The patient underwent laparoscopic right hemicolectomy, and histopathological examination revealed moderately differentiated adenocarcinoma with a mucinous component in the ascending colon tumor.

After obtaining the patient’s informed consent, we analyzed the patient’s tumor and found a high frequency of microsatellite instability. Then, after genetic counseling, we performed genetic analysis using next-generation



sequencing⁶. The assay detected deletion of the *EPCAM* and *MSH2* genes. We conducted further analysis using multiplex ligation-dependent probe amplification (MLPA) to confirm the deletion. Assays were performed to detect large genomic rearrangements of the *EPCAM* and *MSH2* genes using an *MLH1/MSH2* MLPA kit following the manufacturer's protocol (SALSA MLPA KIT P-003 *MLH1/MSH2*, MRC-Holland, Amsterdam, The Netherlands). Then, we detected germline deletions of all *MSH2* exons and exon 9 of the *EPCAM* gene. An additional assay was performed using an *MSH6* MLPA kit (SALSA MLPA KIT P-072 *MSH6*), and we detected the deletion of the *EPCAM* gene and exon 2 of the *KCNK12* gene, which was located upstream of the *EPCAM* gene (Fig. 2a, b).

We identified a novel germline deletion of chromosome 2p16-21, including the *EPCAM*, *MSH2*, and *KCNK12* genes. Large genomic deletions and duplications, such as this one, have recently been identified using MLPA⁵. However, to our knowledge, there are no reports of germline deletions in this region.

The *MSH2* gene is more likely to be found in genomic rearrangements at the locus of this disease because the *MSH2* gene has a higher number and density of Alu repeats than other MMR genes⁷. At least 20% of germline *MSH2* pathogenic variants are deletions of exons or multiple exons.

MSH2 variant carriers have a significantly higher risk of developing cancer of the urinary tract compared with carriers of *MLH1* and *MSH6* mutations⁸. Moreover, male *MSH2* variant carriers have a high risk of developing cancer of the stomach⁹. However, our patient and his relatives had not developed urinary tract cancer or stomach cancer.

The difference in cancer risks between *EPCAM* deletion carriers and *MSH2* variant carriers has been reported. The cumulative risk of endometrial cancer in *EPCAM* deletion carriers is lower than that in *MSH2* variant carriers, while the cumulative risk of colorectal cancer in *EPCAM* deletion carriers is similar to that in *MSH2* variant carriers¹⁰. On the other hand, the cumulative risk of colorectal cancer and endometrial cancer in carriers of a concurrent deletion of the *EPCAM* and *MSH2* genes is reported to be similar to that in *MSH2* variant carriers¹⁰. However, none of our patient's relatives had developed endometrial cancer. In our study, to confirm the role of MMR proteins in carcinogenesis, immunohistochemical staining for *MSH2* and *EPCAM* was conducted on colorectal cancer tissue, which showed only the loss of *MSH2* expression (Fig. 3a, b). Thus, in our patient's colorectal cancer, dysfunction of the *MSH2* protein caused dysfunction of the MMR system, followed by a DNA replication error. Therefore, if the cancer risks were different between the *MSH2* and *EPCAM* genes, the expression of MMR proteins should also be considered.

The *KCNK12* gene located on 2p22-2p21 encodes one of the subfamilies of two-pore-domain potassium channels¹¹. *KCNK12* expression has been found in various organs, such as the pancreas, heart, skeletal muscle, ovary, testis, prostate, colon, peripheral blood leukocytes, small intestine, spleen, and thymus¹¹. By setting and modulating the cellular membrane potential, potassium channels play a significant role in neuronal activity, muscular excitability, and hormone secretion¹². Recent evidence supports the hypothesis that alterations in the expression and function of two-pore-domain potassium channels may contribute to cancer development and progression¹³, including breast cancer, colorectal cancer, leukemia, and lymphoma¹⁴⁻¹⁶. Further, a single-nucleotide polymorphism within the *KCNK12* gene has been identified as a candidate genetic marker for migraine in the Finnish population¹⁷. However, our patient and his relatives did not have migraines or a neurovascular disorder.

The limitations of this report include the absence of segregation analysis and no identification of a breakpoint or rearranged genome. In conclusion, we identified a novel large germline deletion of chromosome 2p16-21,

including the *EPCAM*, *MSH2*, and *KCNK12* genes. However, the clinical significance of germline deletion of the *KCNK12* gene is still unknown. Therefore, it is necessary to obtain and examine more data on this topic.

HGV database

The relevant data from this Data Report are hosted at the Human Genome Variation Database at: <https://doi.org/10.6084/m9.figshare.hgv.2990>; <https://doi.org/10.6084/m9.figshare.hgv.2993>; <https://doi.org/10.6084/m9.figshare.hgv.2996>.

Author details

¹Department of Surgery, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan. ²Department of Clinical Genetics, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan. ³Diagnosis and Therapeutics of Intractable Diseases and Intractable Disease Research Center, Juntendo University Graduate School of Medicine, Tokyo, Japan. ⁴Department of Pathology, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, Tokyo, Japan. ⁵Department of Digestive Tract and General Surgery, Saitama Medical Center, Saitama Medical University, Kawagoe, Japan

Author contributions

All authors contributed to this work. S.N. prepared the description of the clinical course and wrote the manuscript; H.E., Y.O., and H.I. performed the genetic analysis; and T.Y. performed surgery, designed the study, and wrote the manuscript.

Conflict of interest

The authors declare no competing interests.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 10 November 2020 Revised: 9 March 2021 Accepted: 1 April 2021.

Published online: 19 May 2021

References

1. Yamaguchi, T. et al. Accumulation profile of frameshift mutations during development and progression of colorectal cancer from patients with hereditary nonpolyposis colorectal cancer. *Dis. Colon Rectum* **49**, 399–406 (2006).
2. Ligtenberg, M. J. L., Kuiper, R. P., van Kessel, A. G. & Hoogerbrugge, N. *EPCAM* deletion carriers constitute a unique subgroup of Lynch syndrome patients. *Fam. Cancer* **12**, 169–174 (2013).
3. Niessen, R. C. et al. Germline hypermethylation of *MLH1* and *EPCAM* deletions are a frequent cause of Lynch syndrome. *Genes Chromosomes Cancer* **48**, 737–744 (2009).
4. Kuiper, R. P. et al. Recurrence and variability of germline *EPCAM* deletions in Lynch syndrome. *Hum. Mutat.* **32**, 407–414 (2011).
5. Eguchi, H. et al. Identification of a Japanese Lynch syndrome patient with large deletion in the 3' region of the *EPCAM* gene. *JJCO* **46**, 178–184 (2016).
6. Kohda, M. et al. Rapid detection of germline mutations for hereditary gastrointestinal polyposis/cancers using HaloPlex target enrichment and high-throughput sequencing technologies. *Fam. Cancer* **15**, 553–562 (2016).
7. Van der Klift, H. et al. Molecular characterization of the spectrum of genomic deletions in the mismatch repair genes *MSH2*, *MLH1*, *MSH6*, and *PMS2* responsible for hereditary nonpolyposis colorectal cancer (HNPCC). *Genes Chromosomes Cancer* **44**, 123–138 (2005).
8. Joost, P., Therkildsen, C., Dominguez-Valentin, M., Jonsson, M. & Nilbert, M. Urinary tract cancer in Lynch syndrome; increased risk in carriers of *MSH2* mutations. *Urology* **86**, 1212–1217 (2015).
9. Capelle, L. G. et al. Risk and epidemiological time trends of gastric cancer in Lynch syndrome carriers in the Netherlands. *Gastroenterology* **138**, 487–492 (2010).
10. Kempers, M. J. et al. Risk of colorectal and endometrial cancers in *EPCAM* deletion-positive Lynch syndrome: a cohort study. *Lancet Oncol.* **12**, 49–55 (2011).
11. Girard, C. et al. Genomic and functional characteristics of novel human pancreatic 2P domain K⁺ channels. *Biochem. Biophys. Res. Commun.* **282**, 249–256 (2001).
12. Jan, L. Y. & Jan, Y. N. Potassium channels and their evolving gates. *Nature* **371**, 119–122 (1994).
13. Comes, N. et al. Involvement of potassium channels in the progression of cancer to a more malignant phenotype. *Biochem. Biophys. Acta* **1848**, 2477–2492 (2015).
14. Keith, A. D., Zhang, W., Stayner, L. & Argos, M. Associations of two-pore domain potassium channels and triple negative breast cancer subtype in The Cancer Genome Atlas: systematic evaluation of gene expression and methylation. *BMC Res. Notes* **10**, 475 (2017).
15. Kober, P., Bujko, M., Oledzki, J., Tysarowski, A. & Siedlechi, J. A. Methyl-CpG binding column-based identification of nine genes hypermethylated in colorectal cancer. *Mol. Carcinog.* **50**, 846–856 (2011).
16. Gomes, A. Q. et al. Identification of a panel of ten cell surface protein antigens associated with immunotargeting of leukemias and lymphomas by peripheral blood gamma delta T cells. *Haematologica* **95**, 1397–1404 (2010).
17. Nyholt, D. R. et al. A high-density association screen of 155 ion transport genes for involvement with common migraine. *Hum. Mol. Genet.* **17**, 3318–3331 (2008).