A novel MLH1 intronic variant in a young Japanese patient with Lynch syndrome

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Abstract
Lynch syndrome, an autosomal dominantly inherited disease, is characterized by an increased risk of developing colorectal cancer. We found a novel germline variant of MLH1 (IVS6+2T>C) that caused Lynch syndrome in a young Japanese patient who had multiple colorectal cancers. Accurate diagnosis will be highly beneficial in clinical practice for surveillance and genetic counseling of patients and their relatives.

Introduction
Lynch syndrome (OMIM 120435), an autosomal dominant syndrome characterized by cancer predisposition, is caused by germline mutations in DNA mismatch repair (MMR) genes and accounts for 2–4% of all colorectal cancers¹,². Mutation carriers are at risk of early-onset colorectal cancer, endometrial cancer, gastric cancer (particularly in patients from Asian countries, such as Japan and Korea³), and a spectrum of other tumors⁴–⁷.

Genetic testing for these MMR gene mutations is now performed to diagnose Lynch syndrome in clinical practice, so the accumulation of knowledge regarding MMR gene variants is essential. Here, we report a novel germline variant of MLH1 in a Japanese patient with Lynch syndrome.

Case presentation
The patient was a 32-year-old male who was referred for genetic counseling after repeated surgeries for colon cancer. At 29 years old, he developed advanced rectal cancer and underwent robot-assisted laparoscopic intersphincteric resection. The histology of the resected rectal tumor (50 mm in size) indicated adenocarcinoma invading into the fatty tissue beyond the muscular propria. Postoperative surveillance in the following year revealed a non-granular, laterally spreading tumor (LST-NG) in the ascending colon, and he underwent endoscopic submucosal dissection (ESD). This second tumor was mostly limited to the mucosa; however, it partially invaded the submucosa (depth: 520 μm). Permeation into the lymph ducts was positive, and a laparoscopic right hemicolectomy was added to secure curative treatment. The first tumor had partially demonstrated a mucinous carcinoma component (Fig. 1a), and the second tumor showed moderate to poor differentiation within the mucosa (Fig. 1b).

A paternal aunt had developed breast cancer, and his paternal grandfather had gastric cancer at 40 years old. His maternal relatives had no cancer history (Fig. 1c). The patient met the revised Bethesda guidelines⁸, and after providing full informed consent, he was further evaluated by microsatellite instability (MSI) testing and immunohistochemistry (IHC) of MMR protein¹,⁹,¹⁰. MSI analysis, entrusted to FALCO HOLDINGS Co., Ltd. (Kyoto, Japan) and evaluated by five well-known microsatellite markers (BAT25, BAT26, NR21, NR24, and MONO27), demonstrated a high frequency of MSI (5 of 5 markers). IHC of the MMR protein was performed on microwave-retrieved, formalin-fixed, paraffin-embedded sections of his rectal cancer using antibodies specific for MLH1 (Clone ES05,
Dako, Santa Clara, CA, USA), MSH2 (Clone FE11, Dako), MSH6 (Clone FP49, Dako), and PMS2 (Clone EP51, Dako) in accordance with the manufacturer’s recommended protocol (at a dilution of 1:50). IHC revealed a loss of expression of MLH1 and PMS2.

Germline DNA was further analyzed after written informed consent to confirm the diagnosis of Lynch syndrome11,12. DNA was extracted from blood using a QIAamp DNA Blood Kit (QIAGEN, Venlo, Netherlands). Genetic examination was performed by whole-exome sequencing (WES) and by confirmatory Sanger sequencing. WES was conducted using an Ion Torrent AmpliSeq Exome RDY Panel kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer’s recommended protocol13. The Institutional Review Board of Shizuoka Cancer Center approved this study.

A novel heterozygous mutation was detected in the splice donor site of MLH1 intron 6 [NM_000249.3:c.545+2T>C (IVS6+2T>C); localization was Chr3:37050398] and confirmed by the Sanger method (Fig. 1d). This alteration has not been reported previously and was not found in any databases, including ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), the Human Gene Mutation Database (HGMD), and ExAC (http://exac.broadinstitute.org/). A different sequence for a variant at this locus (MLH1 c.545+2T>A) was registered in the HGMD as a disease-causing mutation14. The base sequence of this domain is highly conserved and is reported to be essential for processing normal mRNA15. We designed a primer on intron 6 and performed reverse transcription-polymerase chain reaction (RT-PCR) to confirm the morbidity significance of this variant (Fig. 2a). For RNA extraction, fresh-frozen tissues, which had been stored in liquid nitrogen, were...
submerged in QIAzol Lysis Reagent (QIAGEN) and disrupted using a TissueLyser (QIAGEN). Total RNA was isolated using the miRNeasy mini kit (QIAGEN). RT-PCR was performed using DNase-treated RNA. This RT-PCR produced a normal sized product and abnormal variants that bound intron 6 and intron 7 (Figs. 2b–d). The splicing donor site c.545+2T>C (IVS6+2T>C) caused a splicing abnormality, and this variant was considered to be functionally affected.

The patient developed two colon cancers in a relatively short period at his young age. The initial rectal cancer demonstrated mucinous carcinoma, and the second ascending colon cancer dedifferentiated and permeated into a lymph duct. The National Comprehensive Cancer Network (NCCN) guideline recommends screening of patients with Lynch syndrome for colorectal cancer every 1, 2 years, as their tumors progress rapidly relative to ordinary colorectal tumors. However, patients with Lynch syndrome can occasionally develop subsequent colon cancers within an even shorter interval. At present, an unanswered question is whether this aggressive feature is common in all patients with Lynch syndrome or is limited to those with a specific mutation site. Therefore, clinicians must perform surveillance carefully with these aspects in mind.

In conclusion, we discovered a novel germline variant of MLH1 (IVS6+2T>C) that causes dysfunction of the MLH1 protein and promoted the development of multiple colon cancers in a young patient with Lynch syndrome. Accurate diagnosis of this genetic syndrome is beneficial in clinical practice for genetic counseling and surveillance of these patients and their relatives.
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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

The Institutional Review Board of Shizuoka Cancer Center approved this study.

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References

1. Hampel, H. et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). N. Engl. J. Med. 352, 1851–1860 (2005).
2. Barrow, E., Hill, J. & Evans, D. G. Cancer risk in Lynch syndrome. Fam. Cancer 12, 229–240 (2013).
3. Park, Y. J., Shin, K. H. & Park, J. G. Risk of gastric cancer in hereditary nonpolyposis colorectal cancer in Korea. Clin. Cancer Res. 6, 2994–2998 (2000).
4. Dunlop, M. G. et al. Cancer risk associated with germline DNA mismatch repair gene mutations. Hum. Mol. Genet. 6, 105–110 (1997).
5. Barrow, E. et al. Cumulative lifetime incidence of extracolonic cancers in Lynch syndrome: a report of 121 families with proven mutations. Clin. Genet. 75, 141–149 (2009).
6. Stoffel, E. et al. Calculation of risk of colorectal and endometrial cancer among patients with Lynch syndrome. Gastroenterology 137, 1621–1627 (2009).
7. Kastrinos, F. et al. Risk of pancreatic cancer in families with Lynch syndrome. JAMA 302, 1790–1795 (2009).
8. Umar, A. et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J. Natl Cancer Inst. 96, 261–268 (2004).
9. Shia, J. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part I. The utility of immunohistochemistry. J. Mol. Diagn. 10, 293–300 (2008).
10. Lindor, N. M. et al. Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome: a systematic review. JAMA 296, 1507–1517 (2006).
11. Julie, C. et al. Identification in daily practice of patients with Lynch syndrome (hereditary nonpolyposis colorectal cancer): revised Bethesda guidelines-based approach versus molecular screening. Am. J. Gastroenterol. 103, 2825–2835 (2008). quiz 2836.
12. Pinol, V. et al. Accuracy of revised Bethesda guidelines, microsatellite instability, and immunohistochemistry for the identification of patients with hereditary nonpolyposis colorectal cancer. JAMA 293, 1986–1994 (2005).
13. Shimoda, Y. et al. Integrated next-generation sequencing analysis of whole exome and 409 cancer-related genes. Biomed. Res. 37, 367–379 (2016).
14. Perea, J. et al. Early-onset colorectal cancer is an easy and effective tool to identify retrospectively Lynch syndrome. Ann. Surg. Oncol. 18, 3285–3291 (2011).
15. Shapiro, M. B. & Senathip, P. RNA splice junctions of different classes of eukaryotes: sequence statistics and functional implications in gene expression. Nucleic Acids Res. 15, 7155–7174 (1987).
16. Inoki, K. et al. Depressed-type submucosal invasive colorectal cancer in a patient with Lynch syndrome diagnosed using short-interval colonoscopy. Dig. Endosc. 28, 749–754 (2016).
17. Janvinen, H. J., Meeklin, J. P. & Sistonen, P. Screening reduces colorectal cancer rate in families with hereditary nonpolyposis colorectal cancer. Gastroenterology 108, 1405–1411 (1995).
18. Janvinen, H. J. et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. Gastroenterology 118, 829–834 (2000).