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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 cancers[1,2]. Mutation carriers are at risk of early-onset colorectal cancer, endometrial cancer, gastric cancer (particularly in patients from Asian countries, such as Japan and Korea[3]), and a spectrum of other tumors[4–7].

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 guidelines[8], and after providing full informed consent, he was further evaluated by microsatellite instability (MSI) testing and immunohistochemistry (IHC) of MMR protein[1,9,10]. 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,

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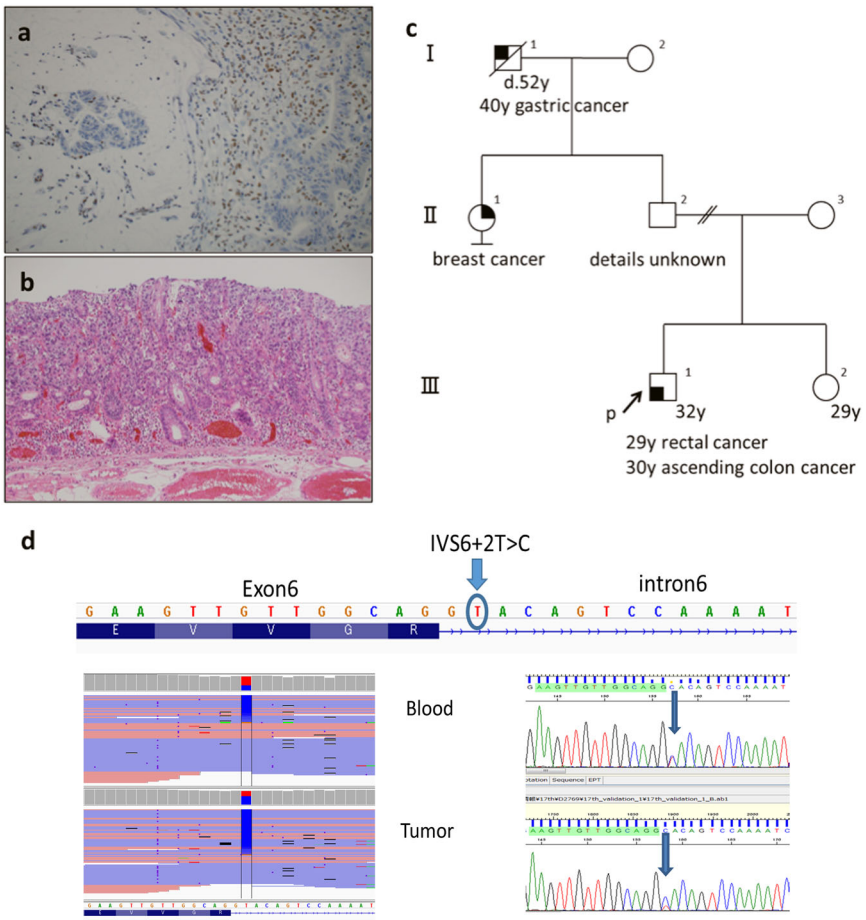

Fig. 1 Pathological view of the colorectal cancers, pedigree, and schematic diagram of a novel *MLH1* mutation. a MLH1 immunostaining of the rectal cancer specimen demonstrated normal expression within the inflammatory cells and interstitial cells, but null expression in the cancer cells, which were found partially floating in a mucinous lake (×200). **b** Pathology of the ascending colon cancer showed moderate to poor differentiation within the mucosa (H&E, ×100). **c** The patient's parents divorced when he was young, and his father's subsequent health condition was unknown. No maternal relatives were cancer sufferers. P proband. **d** A novel heterozygous mutation was detected by whole-exome sequencing in the *MLH1* intron 6 splice site [NM_000249.3:c.545+2T>C (IVS6+2T>C); localization was Chr3:37050398] and confirmed by the Sanger method

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 syndrome[11,12]. 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 protocol[13]. 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): the localization was Chr3:37050398(GRCh37)] (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 mutation[14]. The base sequence of this domain is highly conserved and is reported to be essential for processing normal mRNA[15]. 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

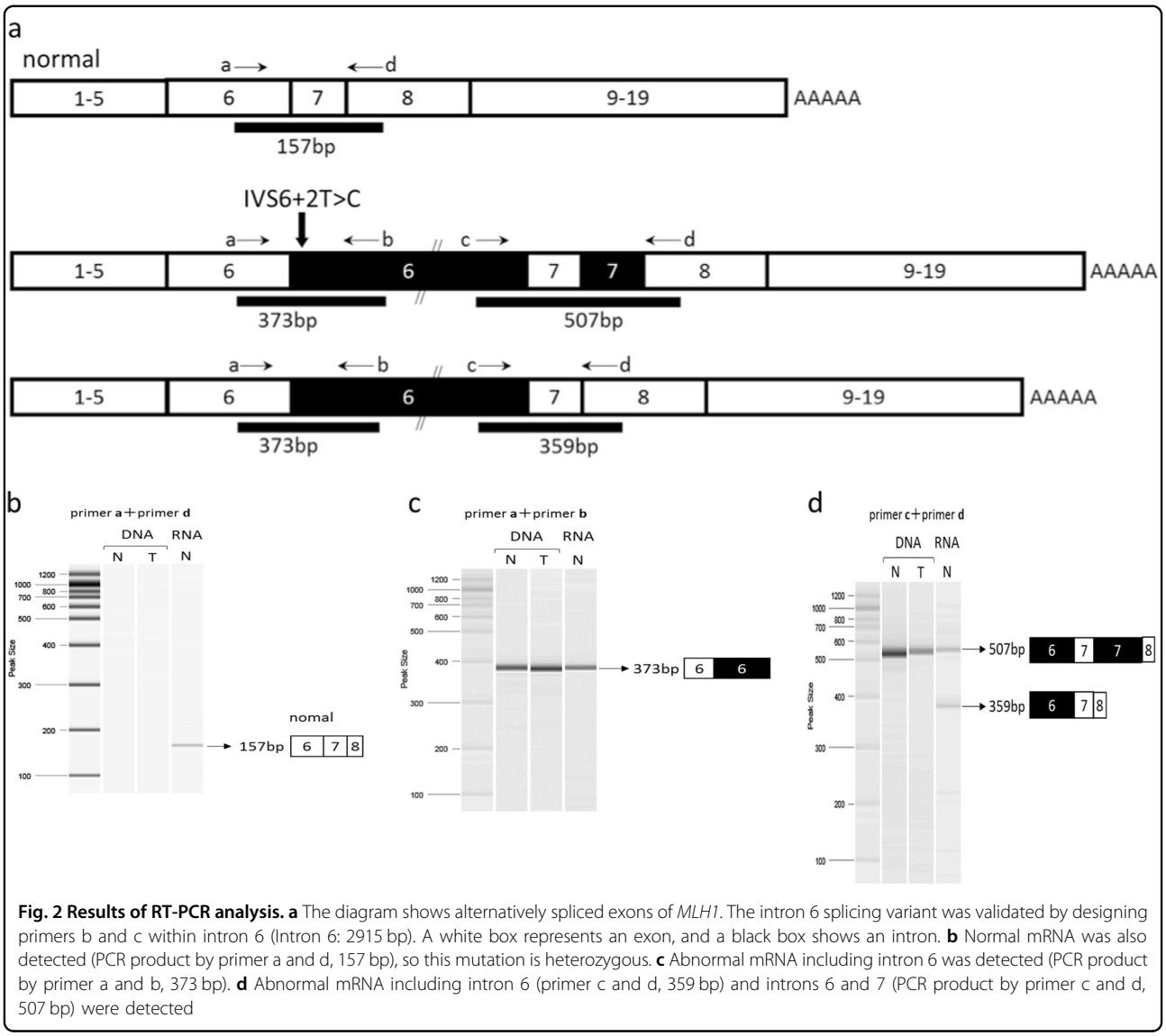

Fig. 2 Results of RT-PCR analysis. a The diagram shows alternatively spliced exons of *MLH1*. The intron 6 splicing variant was validated by designing primers b and c within intron 6 (Intron 6: 2915 bp). A white box represents an exon, and a black box shows an intron. **b** Normal mRNA was also detected (PCR product by primer a and d, 157 bp), so this mutation is heterozygous. **c** Abnormal mRNA including intron 6 was detected (PCR product by primer a and b, 373 bp). **d** Abnormal mRNA including intron 6 (primer c and d, 359 bp) and introns 6 and 7 (PCR product by primer c and d, 507 bp) were detected

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[16]. 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[17,18].

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.1930.

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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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