Case Report
Synchronous gastric and sebaceous cancers, a rare manifestation of MLH1-related Muir-Torre syndrome

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Abstract: Muir-Torre syndrome (MTS), a rare variant of the hereditary non polyposis colorectal cancer syndrome, is an autosomal dominant genodermatosis characterised by coincidence of sebaceous gland neoplasms (sebaceous adenoma, epithelioma, or carcinoma) and at least one internal malignancy. The underlying cause of MTS is a germline mutation in DNA mismatch repair genes MSH2, MLH1 and MSH6. We report the case of a 52-year-old caucasian woman with the development of metachronous colon cancer at the age of 38 years, uterine cancer at the age of 43 years, and unique occurrence of synchronous gastric and sebaceous carcinomas related to germline point mutation c. 2194A>T in the last exon of MLH1 gene, resulting in truncated protein in C-terminal region p. Lys732X due to premature stop codon. This mutation, not previously reported in MTS, disrupts the function of MutL complexes presumably by preventing the interaction with PMS1/PMS2 and impairing the endonuclease active site. This case points out the importance of sebaceous neoplasia, especially sebaceous adenocarcinoma, as cutaneous markers of MTS for timely implementation of cancer screening programs.

Keywords: Muir-Torre syndrome, MLH1, germline mutation, gastric cancer, sebaceous adenocarcinoma

Introduction

Muir-Torre syndrome (MTS) is a rare autosomal-dominant inherited cancer syndrome predisposing to various internal malignancies and sebaceous gland skin tumors. MTS was described in the 1960s by Muir [1] and Torre [2], and subsequently recognized as a rare phenotypic variant of hereditary nonpolyposis colorectal cancer (HNPCC), observed with a frequency of 1% up to 9% in HNPCC patients [3, 4]. MTS is clinially characterized by coincidence of at least one sebaceous gland skin tumor (sebaceous adenoma, epithelioma, or carcinoma) and at least one internal malignancy in absence of other predisposing factors like previous irradiation or immunodeficiency. Molecular genetic studies have reported microsatellite instability (MSI) in the majority of samples from sebaceous and internal tumors, indicating

deficiency in DNA mismatch repair (MMR). In MTS patients, constitutional mutations in MSH2 and MLH1 genes have been identified [5, 6], and more recently few cases with MSH6 mutations were also described [7]. In this study, we report a case of MTS patient, who developed multiple metachronous colon, uterine, gastric and sebaceous carcinomas due to germline nonsense mutation in the last exon of MLH1 gene. This mutation is pathogenic and has not been previously described in association with MTS.

Case presentation

The patient, a 52-year-old caucasian woman with the anamnesis of a colon cancer resected at 38 years (well-differentiated tubular adenocarcinoma, stage I) and consequent uterine cancer resected at 43 years (moderately differ-
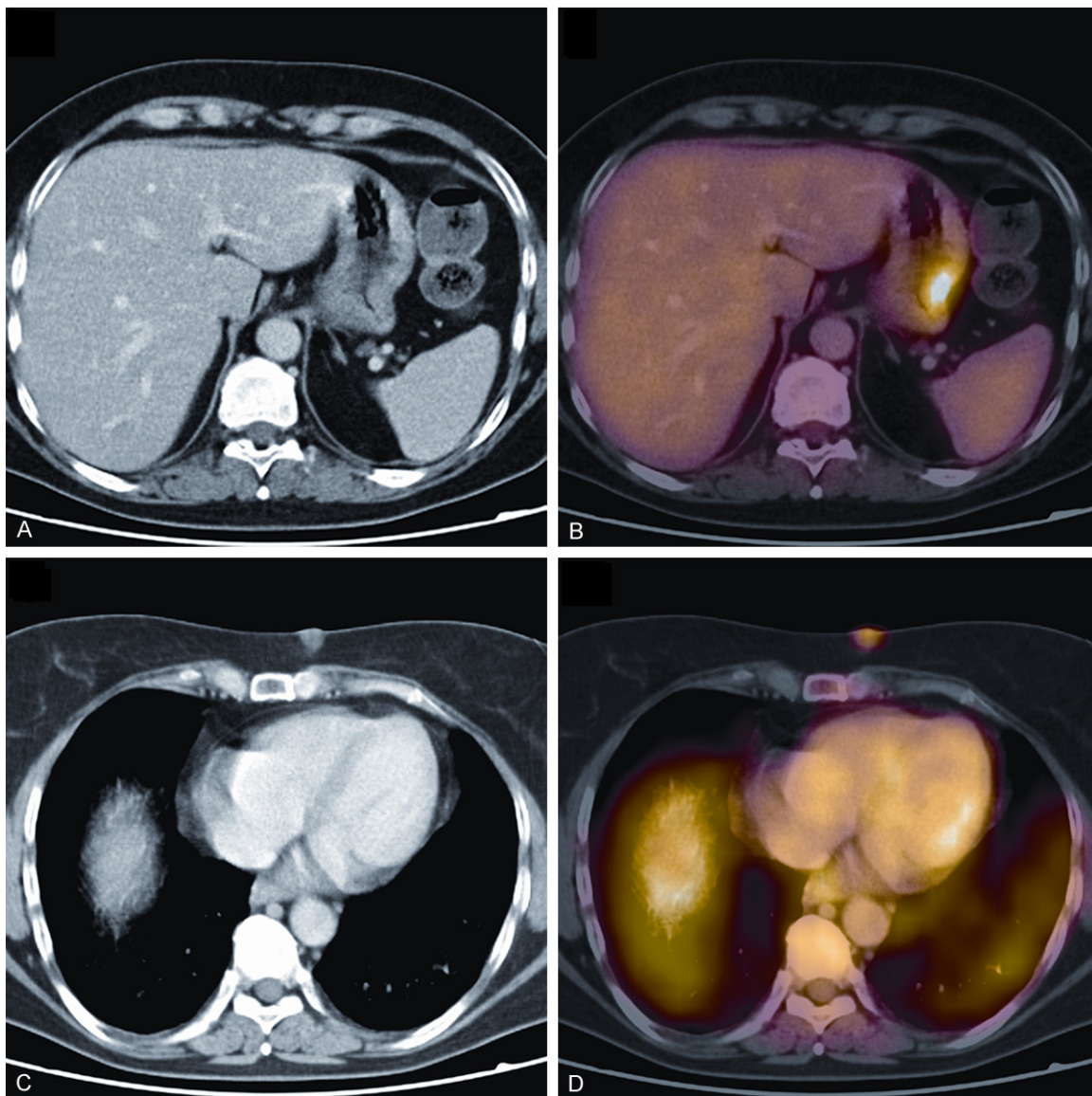
Figure 1. Positron emission tomography-computed tomography (PET/CT) scan at the time of diagnosis. Axial CT (A, C) and fused PET/CT (B, D) views reveal focal thickening of the gastric wall along the greater curvature with corresponding FDG activity (B). Other FDG hyperaccumulation is found in dermal nodule in the left parasternal region (D). Increased FDG uptake is also seen in lower lobes because of residual pneumonia.

enciated endometrioid adenocarcinoma, stage IA), was referred to PET/CT scan for increased CA 15-3 levels to 120 kU/l (normal value 0-35 kU/l). The scan revealed focal pathological hyperaccumulation of fluorodeoxyglucose (FDG) in the gastric wall along the greater curvature, which was markedly thickened measuring 14 mm (**Figure 1A** and **1B**). Another focus of FDG hyperaccumulation was localised at the chest in the left parasternal region, where dermal nodule, approximately 15 mm in diameter, with deep submucosal invasion was located

(**Figure 1C** and **1D**). Both lesions were highly suspicious of viable neoplasia. Subsequent gastroscopy confirmed a polypoid tumor, microscopically assessed as high-grade intraepithelial neoplasia with transition to a well-differentiated tubular adenocarcinoma. The patient was treated with gastric wedge resection. At the same time, microscopic evaluation of a dermal nodule treated with wide local excision revealed a well-differentiated sebaceous carcinoma demonstrating an infiltrative growth within the dermis. Based on the presence of sebaceous
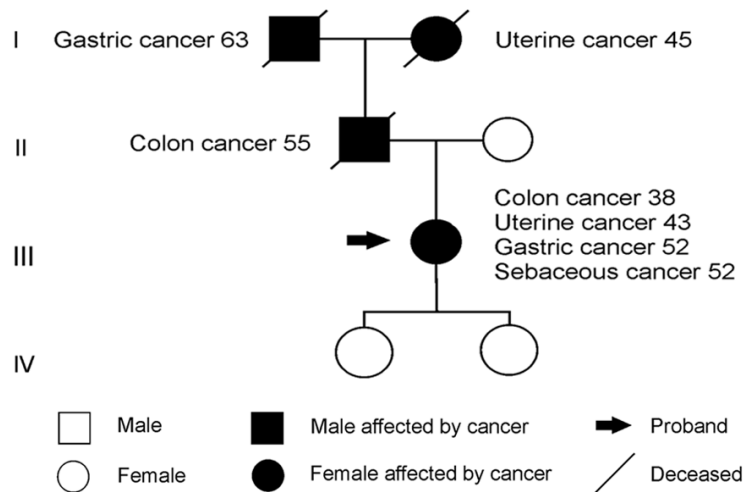
Figure 2. Genealogical tree of the patient's family.

carcinoma and three visceral malignancies, a diagnosis of Muir-Torre syndrome was suggested. Her family history was also significant for multiple tumors (**Figure 2**) and complied with Amsterdam II clinical criteria for identification of HNPCC families [8].

Microsatellite instability and MMR gene mutation analysis

After written informed consent was obtained, genomic DNA was extracted from flash-frozen samples of gastric carcinoma and surrounding normal mucosa, and from peripheral blood by use of the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's standard protocol. MSI status was determined in samples of gastric cancer and normal mucosa using a pentaplex PCR assay of five mononucleotide markers as described by Buhard et al [9]. The tumor tissue showed instability in all five microsatallite repeats (**Figure 3A**), and thus its status was considered as MSI-H. Next we searched for putative germline mutations in MMR genes by use of HNPCC MASTR Assay kit (Multiplicom, Niel, Belgium) covering coding sequences of MLH1, MSH2, MSH6 and the 3' end of EPCAM genes. The PCR products were analyzed by next-generation sequencing using 454 sequencing system on GS Junior platform (Roche-454 Life Sciences, Branford, CT, USA). To verify detected variants, the relevant part of DNA was independently amplified and sequenced by Sanger method. While in MSH2, MSH6

and EPCAM genes no genetic variants were found, two heterozygous mutations were detected in MLH1 gene. The first represented missense mutation c.655A>G, p.Ile219-Val, described as a common polymorphism without functional consequences on MMR capacity [10], whereas the second nonsense mutation c.2194A>T, p.Lys732X, results in premature TAA stop codon and truncated protein (**Figure 3B**). The occurrence of nonsense mutation c.2194A>T was confirmed in two separate blood sample examinations.

Expression of MLH1 protein

In order to verify the impact of MLH1 alteration on protein level, we performed immunohistochemical staining of colon, uterine, gastric and sebaceous tumor tissues using antibody against full-length hMLH1 (clone G168-15, BD Pharmingen, San Diego, CA, USA), which was diluted 1:50 and incubated with the sections at 4°C overnight. All tumor specimens demonstrated loss of MLH1 expression, while stromal and inflammatory cells retained intense nuclear positivity (**Figure 4**).

Discussion

In human cells, MutLα (MLH1-PMS2) are responsible for regulation of excision process by complex interactions mainly with MutS heterodimers, the PCNA replication clamp, the RFC clamp loader, and exonuclease ExoI. In addition, MutLα is a latent endonuclease, which introduces single-strand breaks into a nicked mismatch-containing DNA heteroduplex and thus enables ExoI to remove the strand in a 5'-to-3' direction [11]. Due to the integral part of MLH1 and MSH2 in MMR complexes, it is not surprising that mutations in both genes are the most common findings in patients with HNPCC, where MLH1 germline mutations account for 50% and MSH2 mutations for 39% of all MMR mutations detected [12]. On the contrary, the mutational ratio is inverted among MTS patients with predominant occurrence of MSH2 mutations (86%) as compared to MLH1 ones (11%) [7]. In both genes, the mutations are ran-
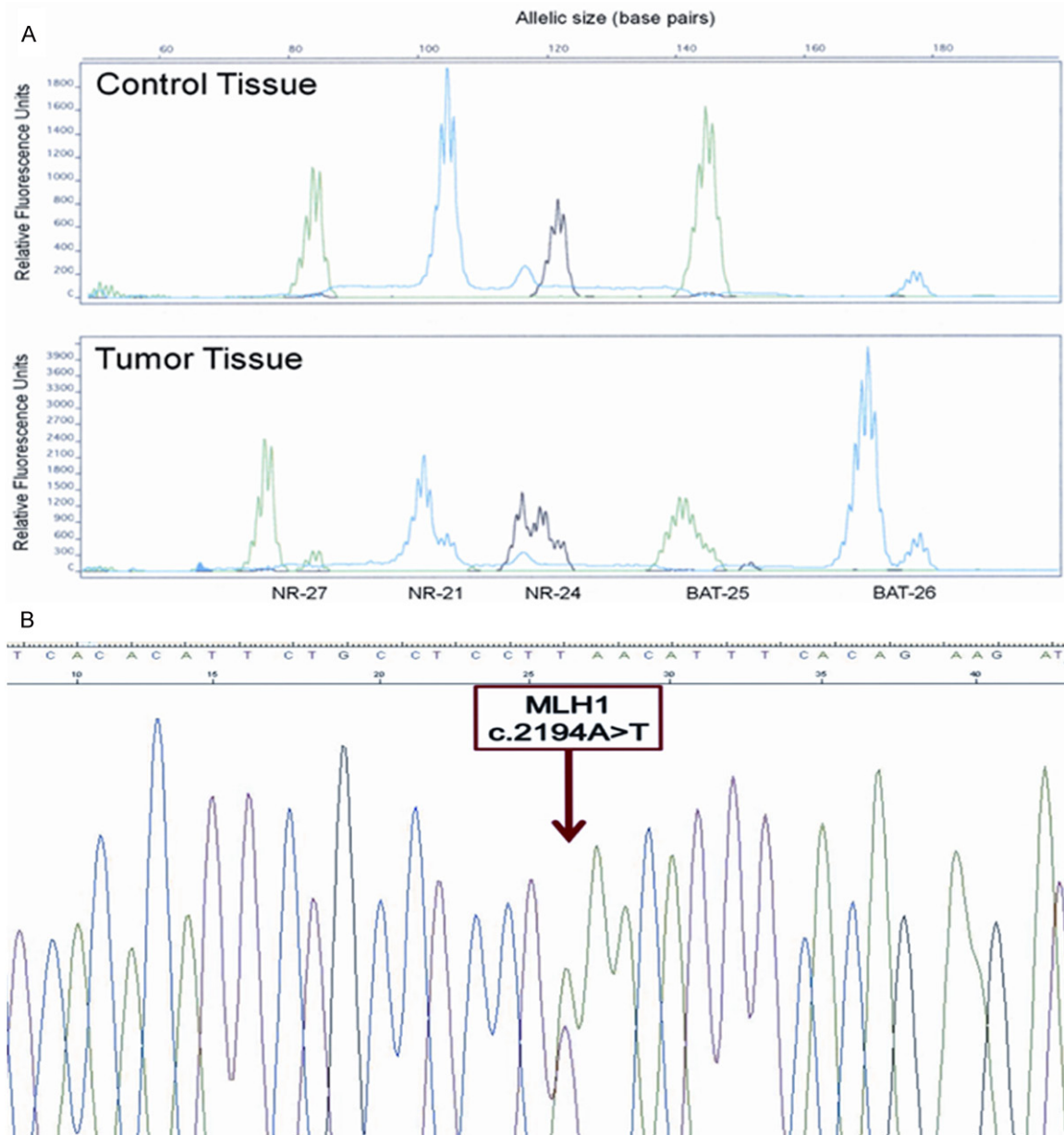
Figure 3. Genetic analysis demonstrating MSI-H gastric cancer and underlying germline mutation in MLH1 gene. (A) Allele length patterns of five mononucleotide markers in control (top) and tumor tissues (bottom); all five microsatellite markers show instability visible as a shortening of the allele length. (B) Detected single-base substitution c.2194A>T in exon 19 of MLH1 gene.

domly distributed with no evidence of a mutational hotspots, neither were reported specific mutations correlating with the MTS fenotype [13].

The molecular genetic analysis of genomic DNA isolated from blood samples of our patient revealed single-base substitution c.2194A>T in exon 19 of MLH1 gene, resulting in premature stop codon (Lys732X) and truncated protein lacking the last 25 amino acids. This germline mutation is pathogenic and has been previously reported only in one Czech patient with HNPCC, but not in association with Muir-Torre syndrome [14]. In the yeast *Saccharomyces cerevisiae*, Mlh1 deletion mutants with 731 amino acid residues and less failed to interact with Pms1 and all truncated variants demonstrated strong mutator phenotype [15]. Recently, the crystal structure of eukaryotic
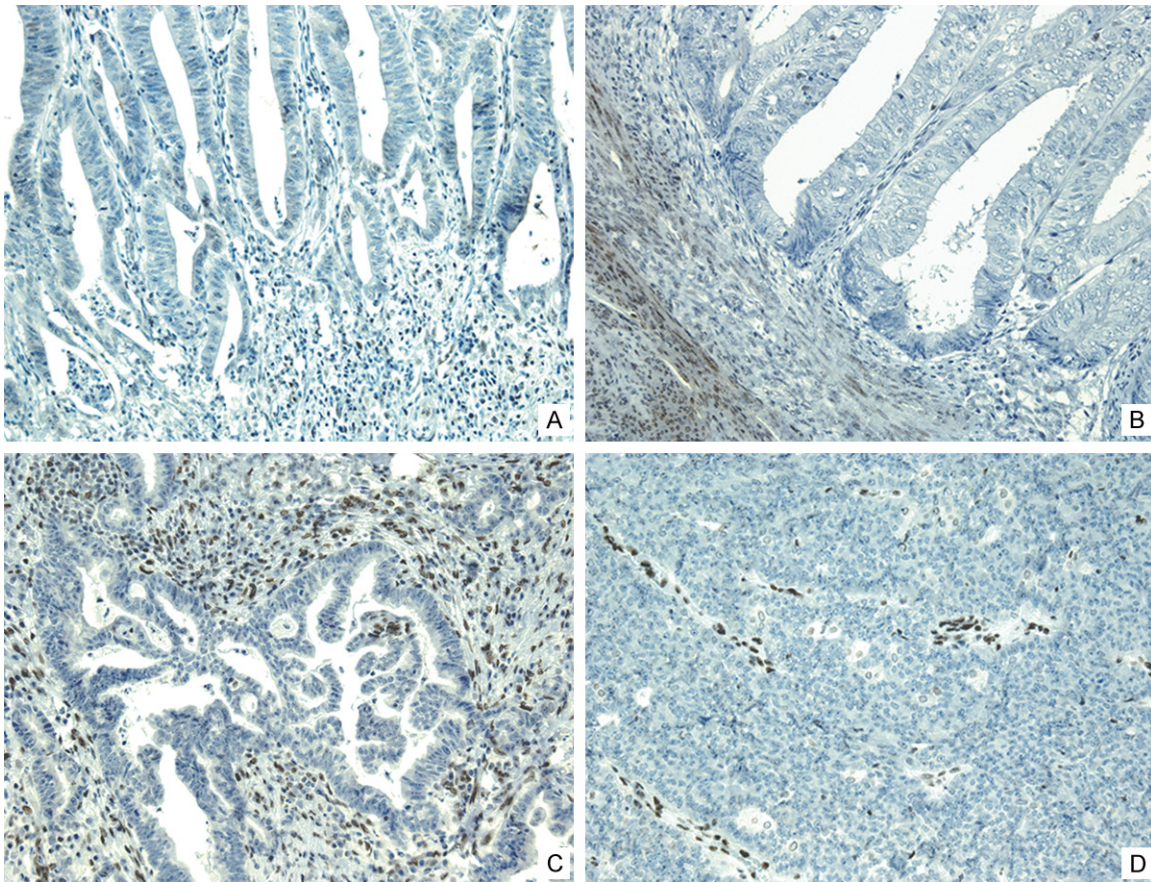
Figure 4. MLH1 immunohistochemical staining. Tumor cells of colon (A), uterine (B), gastric (C) and sebaceous carcinoma (D) displayed loss of MLH1 expression in contrast to strong nuclear staining in stromal and immune cells. Original magnification x100.

MutLα (yeast Mlh1-Pms1 heterodimer) C-terminal domain demonstrated that the conserved C-terminal region of Mlh1 forms part of the Pms1 endonuclease site with direct participation of the last residue Cys769 in Pms1 zinc-binding site. Further functional analysis revealed that Mlh1-ΔC1 variant, lacking cysteine Cys769, binds efficiently to Pms1 but fails in complementation assays, demonstrating more than 10fold higher mutation rate compared to wild-type Mlh1 [16].

Our patient was diagnosed with multiple metachronous carcinomas, including colon, uterine, gastric and sebaceous cancer, and this unusual appearance of tumours is a unique feature of our report. The most common visceral tumor in MTS is colorectal cancer (56%), followed by genitourinary cancers (22%), upper gastrointerstinal tract (5%), carcinomas of the breast (4%), head and neck (3%), and haemato-

logic malignancies [17]. Gastric cancer has rarely been reported in association with MTS [18, 19]. Among 205 patients reported with MTS, only two had gastric cancer [17]. Similar incidence data were found in a large cohort of 6041 members of 261 HNPCC families, where gastric cancer was reported in 1.4% of patients [20].

Sebaceous neoplasms are the characteristic cutaneous markers of MTS and may develop in 22% of cases as the first tumor, concurrently in 6%, and after diagnosis of the internal malignancy in 56% of cases [17]. Among them, sebaceous carcinomas are rare tumors accounting for only 1 out of 2000 of cutaneous malignancies. These tumors are recommended for microsatellite instability testing and immunohistochemical analysis with antibodies against MLH1, MSH2, and MSH6 could indicate the involved MMR gene [3, 21]. In our study, all tumor samples including colon, uterine, gastric,

and sebaceous cancer demonstrated loss of MLH1 expression.

In summary, our case report illustrates the characteristic features of HNPCC family member with unique development of synchronous gastric and sebaceous cancer, complying with MTS variant. The occurrence of sebaceous neoplasia, especially sebaceous cancer, must always raise the suspicion of MTS and prompt a search for associated visceral malignancies. The identification of pathogenic germline mutation is invaluable because of the possibility for genetic testing of other family members and thus could allow the timely implementation of cancer screening program. Fortunately in our case, both daughters of the patient underwent genetic testing and were found negative for reported mutation.

Disclosure of conflict of interest

None.

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