Familial Lynch syndrome with early age of onset and confirmed splice site mutation in MSH2: A case report

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Abstract. Lynch syndrome (LS) is an autosomal dominant cancer syndrome. It can be caused by mutations of several genes, including MLH1, MSH2, MSH6, PMS2, MLH3 and MSH3, which are responsible for DNA mismatch repair, and LS affects 3-5% of patients with colorectal cancer (CRC). LS is associated with a high risk of cancer in several different locations, although the most commonly affected regions are the colon (20-70% risk), endometrium (15-70% risk), stomach (6-13% risk) and ovaries (4-12% risk). In the present report, the familial case of LS with a detected pathogenic variant in the MSH2 gene is described. The proband was a male who was diagnosed with CRC at the age of 25 years. Genealogy analysis revealed a total of seven affected relatives (including the proband), one of whom (I degree relative, mother) had synchronous cancers (endometrial and ovarian) and five others (of II and III degree relation) had ovarian cancer. Genetic analysis using next generation sequencing detected a heterozygous germline mutation in the MSH2 gene (c.1386 + 1G >A) in the proband and his mother, confirming the diagnosis of LS. The results of the recommended genetic test in an asymptomatic relative of the proband (II degree relative, uncle), found the same familial mutation. Subsequent prophylactic colonoscopy of this relative revealed early stage CRC. The presented case confirms the need for specific genetic analysis, alongside genetic counseling, in hereditary cancer syndromes. Active genetic prophylaxis in patients with LS allows early detection of primary cancers in other locations, and pre-symptomatic genetic analysis of relatives is an option for early diagnosis.

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Introduction

Lynch syndrome (LS) is the most common hereditary cause of colorectal cancer (CRC), accounting for 3-5% of all CRC cases (1). LS was previously termed hereditary nonpolyposis CRC (HNPCC) to emphasize the absence of colon polyps and to distinguish it from the other types of hereditary CRC, which are characterized by the presence of polyps, such as Adenomatous Polyposis Coli and Hamartomatous Polyposis syndrome (1). It is estimated that LS is possibly the most common hereditary cancer syndrome, with an overall prevalence of 1/100-1/180 in the general population (1). LS is associated with a high lifetime risk of developing several types of cancer, primarily CRC (20-70% risk with an average age of diagnosis of 44-61 years), endometrial cancer (15-70% risk with an average age of diagnosis of 44-61 years), ovarian cancer (risk 4-12% with an average age of diagnosis of 42.5 years), gastric cancer (risk 6-13% with an average age of diagnosis of 56 years) and for other cancers (small intestine, brain, skin, hepatobiliary and urinary tract, overall risk 15%) (2). The etiology of LS is an inherited germline mutation in one of the Mismatch Repair (MMR) genes-MLH1 (3p22), MSH2 (2p21), MSH6 (2p16), PMS2 (7p22), MLH3 (14q24), MSH3 (5q14), MSH5 (6p21) or MLH2 (2q32) (3). The MMR system is responsible for repairing single base mismatches and small insertions and deletions that occur predominantly during replication (4). According to Knudson's 'two-hit' hypothesis, failure of the MMR repair system is a consequence of bi-allelic inactivation of MMR genes (classical tumor suppressor genes) (3). Therefore, individuals who are carriers of one germline mutation in these genes are simply predisposed to cancer. If the somatic mutation in the second wild-type allele occurs during the carrier's lifetime, a cancer will develop. The somatic mutation in the corresponding wild-type allele is typically a point mutation (3).

A deficiency in the MMR complex leads to a high mutation rate, especially in repetitive DNA sequences (dispersed

sequence elements that make up ~3% of our genome and are usually polymorphic), the so-called microsatellites (MS) (4) This condition is termed MS instability (MSI) and is a specific feature of LS-associated cancers (in ~95% of all cases) (5). Currently, there are two methods to establish the stability of MSs. One is a molecular test that is based on the detection of amplified MS loci by PCR. The analysis is performed on tumor DNA (extracted from tissue embedded in paraffin) and allows the classification of tumor tissues as high MSI (MSI-H)-deficient mismatch repair (dMMR), or low MSI (MSI-L)-MMR functioning properly (6). The other method is based on immunohistochemical (IHC) detection of proteins, encoded by MMR genes (MLH1, MSH2, MSH6 and PMS2). MMR deficiency is defined by loss of expression of some of the four MMR proteins (6). There is no consensus on which of the two tests is preferable for CRC as they have similar performance characteristics in detecting LS (7-10), while for endometrial cancer the preferred test option is IHC, which has a sensitivity of 100% vs. 56.3% for the MSI test, with similar specificity (11,12).

Identification of families with LS is important for the effectiveness of surveillance strategies in affected individuals and the prevention of cancer in their relatives. In clinical practice, there are two major guidelines for identification of individuals and families with LS: The Amsterdam criteria (AC) and the Bethesda guidelines. The AC (adopted in 1990) was used to identify families with CRC eligible for molecular analysis of MMR deficiency (13). Later, these criteria were updated to ACII, including other LS-related cancers (14). It was found that these criteria were very restrictive, resulting in omission of ~68% of patients with LS (15). The second set of guidelines, the Bethesda guidelines, were later developed and expanded the clinical criteria for LS screening, taking into account the MSI status of the tumor tissue. The Bethesda guideline panel includes five MSs-two mononucleotide and three dinucleotide repeats (16). However, even with the updated Bethesda criteria, a large number of patients with LS remain underdiagnosed (17). According to the latest recommendations of the European Hereditary Tumour Group and the European Society of Coloproctology all colorectal and endometrial carcinomas should be tested for MMR deficiency (18). In cases of established MMR deficiency, analysis of a germline mutation in the MMR genes is recommended for precise patient therapy, to improve clinical surveillance and to reduce cancer morbidity and mortality rates in the families of LS patients (18). Knowledge of the molecular mechanisms (type of mutation) and genotype-phenotype correlations enhance the efficiency of genetic counseling in patients with LS. In the present report, the case of familial LS with early onset of cancer and a detected pathogenic splice donor variant in the MSH2 gene is described.

Materials and methods

Patients. The patients (proband and his mother) were referred to the Center of Medical Genetics at the University Hospital 'Dr. Georgi Stranski' (Pleven, Bulgaria) for germline genetic testing. Blood samples were obtained (in an EDTA plastic tube) from the patient and his relatives (mother and uncle) after obtaining informed consent.

IHC procedure. All tumor samples used in the present study were collected after obtaining informed consent for participation in the study. Tumor specimens from the proband's uncle were fixed in 10% buffered formalin for 24-36 h at room temperature, dissected and paraffin embedded. A pathologist selected 5 μm thick parallel sections of representative invasive tumor material and normal mucosa, and the tissue sample was confirmed to contain cancerous tissue using hemoxylin and eosin staining, which was performed as routine. Epitope retrieval time for all tumor sections was 20 min at 97˚C in DAKO PT Link (cat. no. PT100/PT101).

Tumor sections were stained with the following antibodies (all from Dako, Agilent Technologies, Inc., and all came ready to use): ES05-Monoclonal mouse Anti-Human MutL Protein Homolog 1, (cat. no. IR079), FE11-Monoclonal mouse Anti-Human MutS Protein Homolog 2 (cat. no. IR085), EP49-Monoclonal rabbit Anti-Human MutS Protein Homolog 6 (cat. no. IR086), and EP51-Monoclonal rabbit Anti-Human Postmeiotic Segregation Increased 2 (cat. no. IR087) for MLH1, MSH2, MSH6 and PMS2 respectively. Incubation time for all antibodies was 20 min at room temperature. A Dako Agilent Autostainer Link 48 slide stainer was used according to the manufacturer's protocol. The external negative controls were the negative reagent controls in the kit. For internal positive controls, normal colonic mucosa, stromal cells and stromal lymphocytes from the same patients. Results were analyzed manually by a pathologist. Expression was reported as: Normal, (retained expression) nuclear expression in >10% tumor cells and retained expression in the internal control or Negative, (loss of expression) 0% expression in tumor cells and retained expression in the internal control.

Germline pathogenic variant detection. Genomic DNA was isolated from each blood sample using a MagCore Genomic DNA Whole blood kit (MagCore®) according to the manufacturer's protocol. The genetic testing of the proband and his mother was performed by next generation sequencing (NGS). The Trusight Cancer Sequencing Panel (Illumina, Inc.) was used for library preparation according to the manufacturer's protocol. The pan-hereditary cancer panel contained oligo probes targeting 94 genes and 284 SNPs associated with increased cancer predisposition. The procedures were performed following the manufacturer's instructions. Qualified libraries were sequenced on the Illumiina NextSeq 550 platform with a 2x150 bp configuration (Illumina, Inc.). Reads were aligned to the reference human genome hg19. Data output files (gVCF) were imported into BaseSpace Variant Interpreter (Illumina, Inc.). Custom filters (minimum read depth of 20x per variant and excluded silent variants) were created to improve variant annotation and interpretation. The five-tier terminology system of the American College of Medical Genetics and Genomics was used for variant classification (19), including: Pathogenic (P), Likely Pathogenic (LP), Variant of Unknown clinical significance (VUS), Likely Benign (LB) and Benign (B). The variants automatically annotated by the software were manually checked in the primary human genome databases: ClinVar (www.ncbi.nlm.noh.gov/clinvar), dbSNP (www.ncbi.nlm.noh.gov/projrct/SNP) and Ensembl (http://www.ensembl.org).

The familial germline mutation in exon 8 of the MSH2 gene detected by NGS was screened in the proband's uncle

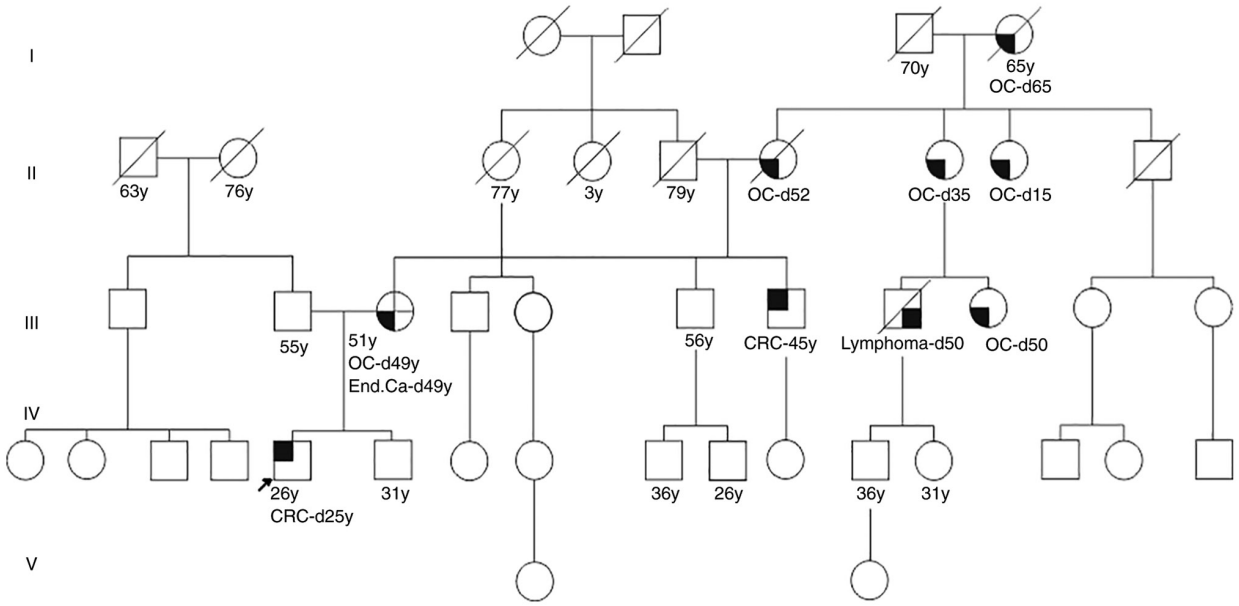

Figure 1. Genealogy of the family. Analysis included 42 individuals from five generations (numbers of generations are indicated with Roman numerals); eight of the family members were affected by a Lynch syndrome-related type of cancer. The proband is indicated by an arrow. Circles are females, squares are males, diagonal slash indicates a deceased individual, the current age/age at death of individuals and the age at diagnosis (indicated with d.) are below the symbols. CRC, colorectal cancer (symbols with filled left upper quadrant); End. Ca, endometrial cancer (symbols with filled right upper quadrant); OC, ovarian cancer (symbols with blanked left lower quadrant).

using direct Sanger sequencing. Primer pairs were designed using the Primer blast tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) to specifically amplify the coding exon 8 of the MSH2 gene and exon-intron boundaries. The primer sequences were: Forward, 5'-GTGGGAAGCTTTGAGTGCTAC-3' and reverse, 5'-ATCCACTGTCCACAAAGGTGC-3'). PCR amplification of the DNA template was performed using AmpliTaq Gold™ 360 Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. Briefly, the reaction mixture consisted of 5 μl AmpliTaq Gold™ 360 Master Mix (2X), 3 μl PCR primers (0.8 μM each) (Applied Biosystem; Thermo Fisher Scientific, Inc.), 1 μl DNA template (10 ng) and 1 μl UltraPure™ DNase/RNase-Free Distilled Water (Applied Biosystems; Thermo Fisher Scientific, Inc.). PCR amplification was run on a GeneAmp™ PCR System 9700 (Applied Biosystems; Thermo Fisher Scientific, Inc.) using the following thermocycling conditions: Initial denaturation at 95˚C for 10 min; followed by 35 cycles of denaturation at 95˚C for 30 sec, annealing at 58˚C for 30 sec and extension at 72˚C for 45 sec; with a final extension step of 72˚C for 5 min. Amplicon sequencing was performed using a BigDye™ Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) and an Applied Biosystems™ 3130xl Genetic Analyser (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol.

Results

The proband (index patient) was a 26-year-old man, diagnosed with metastatic CRC at the age of 25. He presented to the surgical clinic complaining of diarrhea (4-5 times per day) for 8 months, rectal bleeding, fatigue and loss of weight and appetite. Laboratory investigations revealed macrocytic anemia. A CT scan detected an infiltrating rectal tumor (~10 cm) and a hypodense liver lesion (5 mm) as well as mesenteric and iliac lymphadenopathy. Colonoscopy revealed an ulcerative-infiltrative tumor, occupying almost the entire circumference of the rectum. Histopathological examination of endoscopic biopsy specimens indicated moderately differentiated rectal adenocarcinoma. The patient underwent the first surgery under general anesthesia-exploratory laparotomy with deep anterior resection of the rectum with total mesorectal excision with descending rectostomy 'end to end', transverse colonoplasty, temporary/protective ileostomy and cystofix. The patient's second operation was the resection of the liver lesions (1.1 and 0.6 cm), the histopathological examination of which confirmed the preliminary suspicion of colorectal metastasis. A molecular test for MSI was performed on the pathological specimen of the colorectal tumor. The result showed microsatellite instability at 6 of 7 loci. The recommendation to the patient was to undergo germline genetic analysis with sequencing of the MSH2 and MSH6 genes. The patient was referred to our Center of Medical genetics for testing. Genealogy revealed seven relatives with LS-related cancer. The first-degree relative (mother) was diagnosed with synchronous cancers (highly to moderately differentiated endometrial adenocarcinoma and moderately differentiated ovarian cystadenocarcinoma) at the age of 49 and underwent bilateral salpingo-oophorectomy. In addition, five other relatives (II and III degree) were diagnosed with ovarian cancer, two of them with early onset (16 years and 35 years old; Fig. 1).

NGS of the proband and his mother detected a pathogenic variant of MSH2, c.1386+1G >A (NM_000251.3), in both individuals. The genetic counselor's recommendations for the affected individuals (mother and son), in accordance with the NCCN guideline for LS and specifically for MSH2-LS, were a high-quality colonoscopy to be performed and repeated

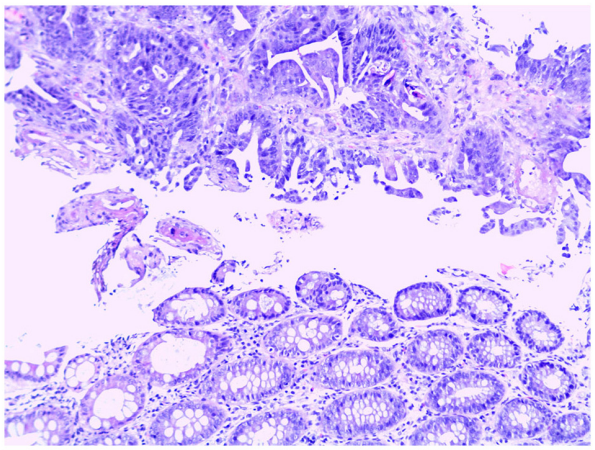

Figure 2. Colonic adenocarcinoma. The biopsy obtained from the proband's uncle showed a moderately differentiated component in the tissue. Samples were analyzed using hematoxylin and eosin staining. Magnification, x4.

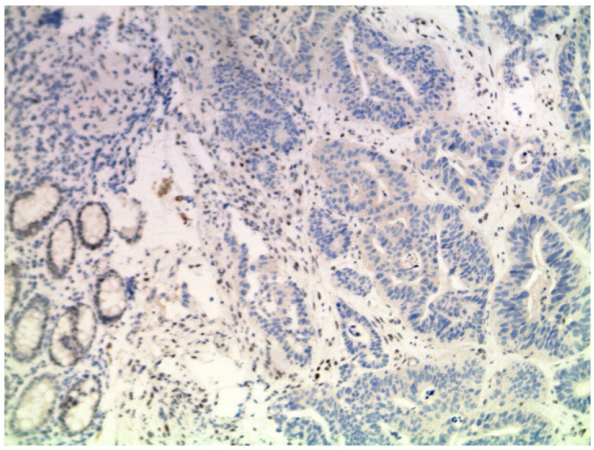

Figure 3. IHC analysis showing the loss of MSH2 nuclear protein expression, performed on the colon tumor tissue of the proband's uncle. Positive internal controls showed preserved nuclear expression in the normal colonic mucosa, stromal cells and lymphocytes. IHC was performed using an anti-MSH2 antibody. Magnification, x10. IHC, Immunohistochemistry.

every 1-2 years (20). Genetic testing for the pathogenic variant c.1386+1G >A in the MSH2 gene was recommended to the I and II-degree relatives of the proband. One of the proband's uncles (45 years old) was aware of his sister's genetic results and underwent a high-quality colonoscopy, which revealed cancer in the flexura coli-hepatica, and was the reason for a laparoscopy-assisted right colectomy. The histological result of tumor formation in the colon showed two components, the first with the morphology of a mucinous adenocarcinoma with extreme extracellular mucus production and ring cells, and the second component of a moderately to poorly differentiated adenocarcinoma (Fig. 2). The tumor tissue was examined for MMR deficiency by IHC and the loss of MSH2 protein expression was revealed, correlating with the carrier status of the familial genetic variant in the MSH2 gene (Fig. 3). Subsequently, the pathogenic variant (c.1386+1G >A) in the MSH2 gene was confirmed by Sanger sequencing in the uncle.

Discussion

LS is one of the most common Mendelian cancer predisposition syndromes, and the resultant cancers are typically localized to the colon and endometrium. LS is associated with other types of cancer locations, including the ovaries, urinary tract, other parts of the digestive system (stomach, small intestine and hepatobiliary tract) and the brain (1,2). The most important criteria (according to Amsterdam criteria) for clinical diagnosis of LS is a family history of colon cancer, although the latest guidelines recommend all patients with colon and endometrial cancer (regardless of their family history) are tested for LS (18). In the present report, the case was not a typical LS family, as the proband had no family history of CRC, but did have a family history of ovarian and endometrial, and this may explain the delayed diagnosis.

The second major clinical characteristic of LS is an early age of onset of the cancer (<50 years old). In the present case, the proband was diagnosed at the age of 25 years, and additionally, two other III-degree relatives (with ovarian cancer) were diagnosed at the age of 16 and 35 years old.

Tumor localization in LS-related CRC occurs with equal frequency in the proximal colon, distal colon and the rectum (21), consistent with the present case. The proband presented with rectal cancer and his uncle with right CRC. Studies have identified the morphological features of CRC specific to LS-related tumors, and include a greater likelihood of a poorly differentiated tumor, a medullary morphology and a mucinous component (22,23), and these features were observed in the case of CRC in the proband's uncle. In contrast, other studies have shown there are no distinguishing features of LS-related CRC (24,25), and this was the case in the proband who presented with a moderately differentiated adenocarcinoma of the rectum, supporting the need for a universal genetic screening panel for LS in all patients with CRC and MSI-H or impairment of MMR genes as assessed using IHC.

LS is caused by a germline mutation in DNA MMR genes, primarily MLH1, MSH2, MSH6 or PMS2 (3). In the present case, the pathogenic variant c.1386+1G >A (NM_000251.3) was found in the MSH2 gene in three of the affected family members. The MLH1 and MSH2 genes (defined as 'major' MMR genes) are crucial for the DNA repair mechanism, and the majority (71 and 84%, respectively) of LS cases are due to germline mutations in these two genes (2,26-28). Amongst all MMR genes, the MSH2 variant is associated with the highest risk of different cancer localizations; although it is typically associated with CRC, as well as endometrial and ovarian cancer (29). In carriers of the MSH2 variant, the cumulative cancer incidence is as follows: 46.6% (females) and 51.4% (males) for CRC, 48.9% for endometrial cancer, 17.4% for ovarian cancer, 18.7% (women) and 17.6% (men) for ureteral and renal cancer, and 23.8% for prostate cancer (30). The pattern of inheritance of MMR pathogenic variants and cancer predisposition is autosomal dominant with a 50% risk for offspring of the affected individual.

In accordance with previous literature, in the described MSH2 familial case of LS, most of the affected members were diagnosed with extracolonic localization of the cancer, in the endometrium and/or ovaries (30).

The reported frequency of the pathogenic germline MMR variants in the general population is as follows: 0.051% (1:1,946) for MLH1 mutations, 0.035% (1:2,841) for MSH2, 0.132% (1:758) for MSH6 and 0.140% (1:714) for PMS2 (31). Amongst all MMR genes, MSH2 mutations have the lowest frequency in the general population, but they are the most common cause of LS due to its major role in the MMR mechanism and the presumed highest penetrance of variants (5).

The most common type of mutation in MMR genes in sporadic CRC and LS differs; however, this comparison between sporadic and hereditary CRC with deficiency of MMR, was not in the scope of the present report. In the described clinical case, the proband had a family history of a I degree relative diagnosed with synchronous cancer (endometrial and ovarian cancer), a clinical feature of LS. Thus, genetic screening for germline mutations in MMR genes was performed. The most common germline mutations in the MSH2 gene are point mutations (nonsense, missense or alterations at the highly conserved splice site position AG/GT) or small insertions/deletions (frameshift) (27,32). The pathogenic variant was found to be c.1386+1G >A (NM_000251.3) in MSH2 (https://www.ncbi.nlm.nih.gov/clinvar/variation/90641/). The variant is located in a canonical splice site and impairs mRNA splicing, resulting in a significantly altered protein as a result of exon skipping, truncation or inclusion of intronic material. Several computational tools predict a significant impact of the variant on normal splicing. Some publications reported experimental evidence showing that the variant leads to exon 8 skipping (33,34). The variant c.1386+1G >A was absent in 248,984 healthy controls (gnomAD); however, it has also been described in multiple individuals with HNPCC (33-35). These data suggest that the variant is likely to be associated with disease. In the present case, this variant was detected in three affected relatives from the family. Taking all the data together, the variant is classified as a pathogenic variant (19).

Diagnosis of LS in the present family was based on the following criteria: Early age of onset of CRC in the proband, synchronous cancer (endometrial and ovarian) in a I degree relative, a strong family history of a LS-related cancer (ovarian), and the detected pathogenic variant in the MSH2 gene. The other affected family members were not available for testing, but their early age of onset was suggestive of their carrier status. When interpreting the test results during genetic counseling, consideration should be given not only to the detected genetic mutation, but also the personal and family history of the patient.

In the family described, there was no need for additional recommendations for the proband and his relative II-degree relative, as they had already undergone colorectal surgery. For the proband's mother, a high quality colonoscopy (repeated in 1-2 years) was recommended. All unaffected family members at risk were advised to undergo genetic testing and, in the case of confirmation of being a carrier of the MSH2 variant, to undergo a high-quality colonoscopy at the age of 20 years (5 years earlier than the age at which the cancer occurred in the proband). Female relatives at risk were advised to be alert for any abnormal uterine bleeding or postmenopausal bleeding (uterine cancer can be detected early by symptoms), and about prevention of ovarian cancer (due to the late onset of clinical symptoms) in MSH2 carriers, bilateral salpingo-oophorectomy should also be considered. Usually the timing of risk-reducing surgery should take into account reproductive history, menopausal status, comorbidities and family history.

Due to the positive family history of ovarian cancer at an early age and the familial pathogenic MSH2 variant (which is associated with a particularly high risk of ovarian cancer), prophylactic surgery should be performed as early as possible. In women with incomplete reproduction, ovarian cancer prevention should include transvaginal ultrasound screening along with a serum test for CA-125.

The presented familial cancer case with the detected pathogenic variant in the MSH2 gene may contribute to genotype-phenotype correlation in LS cases. Carriers of a splice site mutation in MSH2 with a very early age of onset of CRC were identified in the present study. The unusual presentation in the family-predominantly with ovarian cancer-is consistent with previous literature showing that MSH2 mutation carriers had a higher preponderance of extracolonic tumors. The early detection of CRC in the uncle of the proband emphasizes the role of genetic counseling in cases of hereditary cancer syndrome-for prevention and effective surveillance strategies.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the ClinVar repository (https://www.ncbi.nlm.nih.gov/clinvar/submitters/507074/).

Authors' contributions

SLP recruited the patients. ZBK, SLP, KSK and KTP collected clinical and biological data. ZBK and SEN performed the molecular analysis. SLP was responsible for diagnosis and treatment of patients. ZBK analyzed the data. ZBK, SLP and KSK were involved in the writing and revision of the manuscript. SEN and KTP reviewed and revised the manuscript. All authors have read and approved the final manuscript. ZBK and SLP confirm the authenticity of all the raw data.

Ethics approval and consent to participate

This study was approved by the Ethics Commission of the Medical University-Pleven (Pleven, Bulgaria. All the participants in the study were >18 years of age and provided written informed consent to participate in the study.

Patient consent for publication

Written informed consent for publication of their data was obtained from all patients.

Competing interests

The authors declare that they have no competing interests.

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