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CASE REPORT

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Double frameshift mutations in *APC* and *MSH2* in the same individual

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Abstract Heterozygous germline DNA mismatch repair gene mutations are typically associated with HNPCC. Here we report the case of a proband whose father was known for familial adenomatous polyposis. The number of polyps (<10) was not typical of polyposis, therefore the diagnosis of HNPCC was entertained. Microsatellite instability analyses were performed on peripheral blood and biopsy of a right-sided dysplastic adenoma. The tumour tissue showed high-grade instability and subsequently immunohistochemistry showed that neither MSH2 nor MSH6 proteins were expressed in tumour cells. Prophylactic colectomy was performed and an adenocarcinoma developing within the adenoma was diagnosed (pT1N0). Genomic DNA analysis revealed a novel mutation in *MSH2* as: a frameshift mutation in exon 7 (c.1,191_1,192dupG). Both parents of the proband were analyzed for *MSH2* and *APC* mutations, and in the father a truncating mutation in exon 15 of *APC* was identified as del3471-3473GAGA. This mutation was found to be present in the proband. His mother was found to bear the *MSH2* exon 7 mutation. At follow-up, the proband was diagnosed with fundic, antral and duodenal adenomas (one fundic adenoma showed low-grade dysplasia). Several tubular rectal adenomas with low-grade dysplasia were excised. The patient later developed an intra-abdominal desmoid tumour.

Keywords Hereditary colorectal cancer · Mismatch repair · Familial adenomatous polyposis · Hereditary nonpolyposis colorectal cancer · Microsatellite instability

Abbreviations HNPCC: hereditary nonpolyposis colorectal cancer · MMR: mismatch repair · FAP: familial adenomatous polyposis · AFAP: attenuated familial adenomatous polyposis · CRC: colorectal cancer · MSI: microsatellite instability · IHC: immunohistochemistry · ACF: aberrant crypt foci · NF1: neurofibromatosis type 1

Introduction

Among the known genetic predispositions to cancer syndromes, two confer a high risk of colorectal cancer (CRC). Familial adenomatous polyposis (FAP), generally due to mutations in the *APC* gene on chromosome 5, is responsible for approximately 1% of all CRC. Mutations in at least four mismatch repair (MMR) genes, *MLH1*, *MSH2*, *MSH6* and *PMS2*, result in Lynch syndrome, or HNPCC (hereditary nonpolyposis colorectal cancer); together these MMR gene mutations account for 3–5% of all CRC [1–3]. Given the relatively high incidence of these conditions, one would expect an occasional individual to be homozygous for the two *APC* or two MMR mutations, or to be a carrier of compound heterozygous germline mutations in *APC* and MMR genes [4–9] Table 1.

To our knowledge however, the present case is the first patient observed with concomitant inheritance of pathogenic frameshift mutations in *APC* and *MSH2*.

We report here a complete description of this family with 5-year follow-up of the proband. As he presented fewer polyps than expected at age 25 in a patient with FAP: the differential diagnosis was between HNPCC and attenuated FAP (AFAP) [10, 11].

Case report

The proband, a 24-year-old man, consulted because of a history of FAP in his father and at least seven other members of his southern Italian family (Fig. 1). His father's medical record was not available when this study begun and no genetic testing had been performed. Five polyps were detected on ileocoloscopy, two in the right colon and three in the sigmoid; four of the polyps were adenomas, one of which was dysplastic.

Table 1 Summary of reported families with *APC* and MMR germline mutations

References	Genes involved	Clinical findings
Scheenstra et al. (2003) [9]	*APC*, *MLH1*	10-year old with symptomatic FAP, rapid progression of adenomas
Yuan et al. (1998) [7]	*APC*, *MLH1*	*APC* polymorphism I1307K; family not of Jewish origin; does not segregate with HNPCC disease phenotype
Yuan et al. (1999) [8]	*APC*, *MSH2*	*APC* polymorphism I1307K; Ashkenazi Jewish individual; variant does not segregate with HNPCC disease phenotype
Present case (2004)	*APC*, *MSH2*	Right-sided polyps (AFAP), but early age of CRC (24), gastric and rectal adenomas with dysplasia

As the number of polyps was not typical of polyposis, the diagnosis of HNPCC was entertained. Microsatellite instability analysis was performed on DNA from dysplastic adenoma and from peripheral blood. The tumour tissue showed high-grade instability (MSI-H) at all four markers tested: BAT25, BAT26, D2S123 and D5S346. IHC on four DNA mismatch repair proteins was also performed. MSH2 and MSH6 were not expressed in tumor cells, whereas MLH1/PMS2 expression was conserved.

Genetic analysis started with *MSH2* and bidirectional sequencing of the exons and the intron–exon junctions using amplification products obtained by PCR from genomic DNA. A novel mutation was identified in *MSH2*: insertion of a G in codon 398, in exon 7 at position 1,191 (c.1,191_1,192dupG), had created a premature termination 19 codons downstream.

The patient opted for prophylactic surgery at age 25 and underwent total colectomy with ileo–rectal anastomosis. In the prophylactic colectomy specimen, a small well-differentiated adenocarcinoma (pT1N0), developing from a tubulo–villous adenoma was found in the right colon, along with "multiple" flat adenomas and associated aberrant crypts. No intra-abdominal desmoid tumour was found at this time.

Over the last 4 years, the patient has experienced numerous complications: soon after colectomy, he had frequent episodes of gastro–esophageal reflux which were temporarily lessened by a proton-pump inhibitor (pantoprazol). At the age of 25 oeso–gastroduodenoscopy showed chronic gastritis (negative for *Helicobacter pylori*), hiatal hernia and approximately 30 fundic polyps. Out of three adenomas excised from duodenum, antrum, and gastric fundus, the latter showed low-grade dysplasia. Rectoscopies were carried out at 3-month intervals over the following year, and showed the presence of two to nine polyps, at least one of which was a tubular adenoma with mild dysplasia. The patient was placed on rofecoxib in an attempt to reduce polyp development, but micro-perforations led to peritonitis and emergency surgery. On this occasion, a mesenteric desmoid tumour was diagnosed from biopsy. To eliminate the risk of additional rectal cancer a proctectomy with endo-anal mucosectomy, and implementation of a pelvic pouch procedure was proposed, but was declined by the patient.

Bi-annual rectoscopy and esogastroduodenoscopy are currently carried out as surveillance. At his most recent examination (at age 28), the proband presented with fewer than ten rectal polyps (one serrated adenoma) but presented multiple gastric fundic polyps (excised polyp was adenoma with low-grade dysplasia).

At the time of their son's surgery for colectomy both parents consulted. As already mentioned, the father presented with familial adenomatous polyposis (FAP); he had undergone colectomy at age 53 after FAP with colon carcinoma was diagnosed. An *APC* gene mutation search was initiated in our laboratory. PTT detected a truncating mutation in exon 15, then caracterized as c.del3471_3474-GAGA. This frameshift mutation creates a premature stop

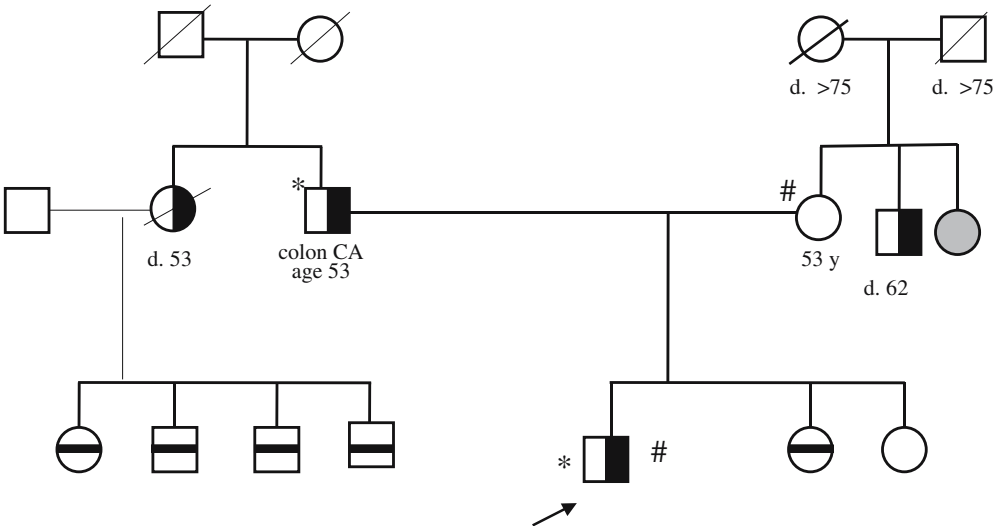

Fig. 1 Proband's family pedigree with *APC* and *MSH2* germline mutations. Pedigree of the family segregating an *APC* mutation (father of proband) and *MSH2* mutation (mother of proband). Half-filled symbols indicate colon cancer, filled gray symbols endometrial cancer and horizontal lines clinical polyposis coli. Proven *APC* mutation carriers have an * and proven *MSH2* mutation carriers an #. The arrow indicates the proband

codon six codons downstream. His son (the proband), carrying the *MSH2* exon 7 mutation, was found to have inherited the *APC* exon 15 mutation from his father. The *MSH2* mutation was absent in the father.

We then analyzed the proband's 53-year-old mother, who had no significant medical history but had not undergone colonoscopy, carries the *MSH2* mutation. Clinical screening for digestive and gynecological pathologies was recommended. Of her seven siblings, a brother reportedly died of colon cancer in his fifties and a sister of uterine cancer at age 62. Genetic testing was offered to all at-risk family members.

Discussion

At the age of 25, the proband's clinical presentation was more suggestive of HNPCC or possibly AFAP than of classical FAP, on both clinical and histological grounds. This observation, along with the lack of confirmatory medical records about his father, led us to perform screening tests (MSI, IHC) for HNPCC. The proband was found to harbour two germline mutations predisposing to CRC: an *APC* exon 15 frameshift mutation inherited from his father and a frameshift mutation in *MSH2*, inherited from his mother.

Upon further evaluation, the proband showed clinical characteristics of both disorders: desmoid tumours are indicative of FAP, and the small number of polyps in the proximal colon (less than ten) was suggestive of AFAP or HNPCC. His clinical course has, however, been relatively severe, with an adenocarcinoma of the right colon diagnosed at age 25, and the postoperative development of a mesenteric desmoid tumour. Desmoid tumours occur in 80% of individuals with FAP who undergo prophylactic colon surgery [14]. Several studies have demonstrated an increased risk for desmoid tumours, in patients with *APC* mutations occuring between codons 1,310 and 2,011 [12–14]. In our patient, the *APC* mutation is located at codon 1,157 and there is no family history of desmoids in this kindred.

Gastric adenoma and/or cancer has been described in both AFAP and HNPCC [15–17]. *Helicobacter pylori* infection may induce atrophic gastritis in FAP patients and trigger adenoma development, but our patient tested negative for *Helicobacter pylori*. As gastric cancer/dysplasia is part of the HNPCC tumour spectrum, *MLH1* and *MSH2* mutations may influence gastric cancer progression.

A more aggressive clinical course has been reported in the handful of reported patients with both *APC* and a second predisposing mutation in a different gene. Zajac and colleagues [18] reported an 18-year-old with inherited FAP and >1,000 polyps. The familial mutation, upstream from the mutation cluster region in the *APC* gene, had not been associated with a particularly severe phenotype in this family. Because of the proband's clinical severity a search for additional mutations was undertaken and a germline *p53* mutation identified. The authors hypothesized that the mother, who died at age 28 of uterine carcinoma, may have also had double mutations.

The only other patient reported to harbor concomitant mutations in *APC* and a MMR gene was a 10-year-old child with symptomatic polyposis; the *APC* mutation was a maternally-inherited prevalent frameshift in exon 15, and the paternally-inherited MMR mutation was a splice site mutation in *MLH1* [9]. The authors hypothesized that the rapid progression to high-grade adenomas in this patient was due to the influence of the *MLH1* mutation; high-grade dysplasia was associated with loss of MLH1 expression by IHC criteria. Surely, the severe phenotype may simply be a reflection of the clinical variability seen in both FAP and HNPCC.

It is worth noting that in mice that are compound heterozygotes for *APC* and *MLH1* germline mutations, cancer phenotypes are also enhanced [19, 20].

Two families have been described [7, 8] with an MMR gene mutation plus the *APC* missense variant I1307K. The latter change, present in 6% of the Jewish population, does not result in FAP, but in some individuals appears to predispose to colorectal polyps, as a possible result of hypermutability of the altered region of the *APC* gene; the relative risk of CRC with the I1307K polymorphism has been reported as 1.5–2.0 [21]. In the first of the two families, a French Canadian CRC kindred with an *MLH1* truncating mutation, there was no clear association between the *APC* I1307K polymorphism and the development of cancer [7]. The same was true in a second such family, of Ashkenazi origin, which fulfilled the Amsterdam criteria for HNPCC. Affected individuals had a missense mutation of *MSH2*, which was later proven to be pathogenic [22]; however, presence or absence of the *APC* I1307K genotype did not correlate with disease. Thus, in these two families, one clear mutation (in a MMR gene which correlated with the HNPCC phenotype) was defined, whereas the *APC* polymorphism was transmitted independently of the CRC phenotype.

Kinzler and Vogelstein [23] proposed that the *APC* gene serves as the "gatekeeper" of epithelial proliferation, thus exerting a major influence on initiation of the neoplastic process. By contrast, the MMR mutations underlying HNPCC alter "caretaker" genes, and would accelerate carcinogenesis, once the initiation step has occurred. However, the distinction between gatekeeper and caretaker roles has recently become somewhat blurred, as the mutation of *APC* could not be shown to be a compulsory step in all CRC. Indeed, a significant proportion of colorectal cancer may be initiated through the epigenetic silencing of alternative genes implicated in apoptosis and mismatch repair [24]. In dysplastic microscopic epithelial lesions referred to as aberrant crypt foci (ACF), flat tubular adenomas, and polypoid tubular adenomas, the frequency of *APC* mutations is reported to be 0, 7 and 36%, respectively [25]. Moreover, genomic instability has actually been detected in ACF. These observations led to two contrasting hypotheses: a "dysplasia ACF–adenoma–carcinoma sequence", or a "heteroplastic ACF–adenoma–carcinoma sequence". These observations may suggest the *APC* mutation consistently triggers adenoma formation before the *MSH2* mutation participates in tumorigenesis through MSI. Molecular analysis of ACF or the demonstration that very early adenomas show abnormal expression of beta-catenin [26], should help to clarifiy this issue.

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