SHORT REPORT

Duodenal carcinoma in MUTYH-associated polyposis

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J Clin Pathol 2006;59:1212–1215. doi: 10.1136/jcp.2005.031757

Bi-allelic germline mutations in the *MUTYH* gene give rise to multiple adenomas and an increased incidence of colorectal cancer. In addition, duodenal adenomas and other extra-colonic manifestations have been described in *MUTYH*-associated polyposis (MAP) patients. We describe two patients with bi-allelic *MUTYH* gene mutations with duodenal carcinoma. The tumour in Patient A was detected during evaluation of non-specific abdominal complaints. Patient B was already diagnosed with tens of adenomas and a colon carcinoma, when a duodenal neoplasm was detected. The identification of somatic G>T mutations in codon 12 of the K-RAS2 gene provides evidence that the duodenal lesions were induced by *MUTYH* deficiency. Studies in larger series of MAP patients are needed to investigate the risk of upper-gastro-intestinal malignancies and to determine further guidelines for endoscopic surveillance.

Patients with homozygotic or compound heterozygotic germline mutations in the *MUTYH* gene are predisposed to develop multiple colorectal adenomatous polyps. Polyp counts vary between 10 and 500 in most cases and approximately 60% of the patients develop colorectal carcinoma.^{1–4} *MUTYH*-associated polyposis (MAP) is clinically comparable to attenuated familial adenomatous polyposis (AFAP) with respect to a relatively late age of diagnosis, a variable number of adenomas and a low incidence of colonic manifestations.

Only a few patients with MAP, with duodenal adenomas, and one patient with stomach cancer have been described.^{4–5} Recently, we reported five patients with MAP with upper gastrointestinal lesions, one of whom developed duodenal carcinoma.⁶ Here we describe the case of duodenal carcinoma in detail, of a 65-year-old man presenting with upper abdominal pain, and a second patient with MAP with duodenal carcinoma who was under surveillance because of multiple colorectal and upper gastrointestinal polyps.

CASE REPORTS

Case A

Patient A had irritable bowel syndrome for several years. Upper gastrointestinal endoscopy at 63 years had not shown any abnormalities. He presented at age 65 with upper abdominal pain. Plain abdominal radiographs showed signs of coprostanis, and flexible sigmoidoscopy showed at least 15 polyps up to 1.5 cm in size localised in the rectum and sigmoid. Histopathological examination showed villous adenomas with low-grade dysplasia. Subsequently, the abdominal pain worsened and the man had vomiting due to gastric food retention. A computed tomography scan suggested small bowel invagination at the junction of the duodenum and jejunum. Emergency laparotomy was carried out and

showed a circular adenocarcinoma at the level of Treitz ligament, which was completely resected. Histopathological examination showed a moderately differentiated adenocarcinoma (fig 1) with a diameter of 6 cm, resection margins free of tumour and no lymphnode metastasis. Remarkably, no other adenomas were seen in the removed duodenum. Further colonoscopy 1 month later showed multiple polyps. Histopathological examination of three polyps showed villous and tubulovillous adenomas with low-grade and high-grade dysplasia. Subsequently, the patient underwent a total colectomy. Histological examination of the resected colon showed many adenomas and three moderately differentiated mucinous adenocarcinoma located in the caecum and ascending colon. In addition, seven highly dysplastic adenomas were found in the descending colon.

Surveillance endoscopy 1 year after the resection of the colon and part of the small intestine again showed an exophytic, circular tumour in the duodenum, whose endoscopic appearance was highly suggestive of malignancy. Biopsy specimens of the tumour showed adenoma containing high-grade dysplasia. A computed tomography scan of the upper abdomen showed no further abnormalities. The decision was made to carry out a Whipple resection. Histological examination of the resection specimen showed one tubulovillous and one villous adenoma with high-grade dysplasia without signs of invasive growth.

The patient's family history included three siblings: one sister with breast cancer at age 40 years, a brother with renal cancer (age unknown) and a brother who died at age 70 years from the consequences of chronic obstructive pulmonary disease.

Case B

In 1993, at the age of 46 years, patient B had undergone a right hemicolectomy for colorectal cancer (CRC; Dukes' B). Apart from the cancer, multiple tubular adenomas had been found in the resection specimen. Between 1993 and 2003, five surveillance colonoscopies had been carried out, during which approximately 15, mostly small, adenomas had been removed. Histological examination showed tubular, tubulovillous and villous adenomas with low-grade dysplasia in most cases, although focal high-grade dysplasia was found in a small, flat villous adenoma.

In 2003, magnifying chromoendoscopy showed numerous (approximately 75) small, raised lesions with abnormal pit patterns (fig 2). Histological examination showed tubular adenoma with low-grade dysplasia. Subsequently, during upper gastrointestinal endoscopy a tubular adenoma with low-grade dysplasia of approximately 15 mm was removed.

Abbreviations: AFAP, attenuated familial adenomatous polyposis; CRC, colorectal cancer; FAP, familial adenomatous polyposis; MAP, *MUTYH*-associated polyposis; PCR, polymerase chain reaction.

Figure 1 (A) Patient A: Small intestine with adenocarcinoma invading deep into the muscular wall (muscularis propria); haematoxylin and eosin, magnification 2 $\times$. (B) Patient B: Duodenal adenoma showing high-grade dysplasia. No infiltrative cells are discernible in this biopsy material (appearing endoscopically as a carcinoma), haematoxylin and eosin, magnification 50 $\times$. (C) Patient B: Supraclavicular lymph node metastasis. Pre-existent lymph node tissue is not visible, haematoxylin and eosin, magnification 50 $\times$.

In the corpus of the stomach three hyperplastic, inflammatory polyps without signs of neoplasia were removed.

On the basis of the results of the magnifying endoscopy and the patient's CRC history, the decision was made to carry out a subtotal colectomy of the remaining colon, with the creation of an ileorectal anastomosis. Histological

Figure 2 Magnified chromoendoscopic view of colonic mucosa of patient B showing multiple small, slightly raised lesions with abnormal pit patterns (Kudo type IIIs) compatible with tubular adenomas.

examination of the resection specimen showed numerous (between 50 and 100) small and very small tubular adenomas with low-grade dysplasia but no high-grade dysplasia or invasive carcinoma. About 7 months after surgery, the patient developed a left subclavian vein thrombosis due to compression by an enlarged lymph node. Cytological examination showed metastasis of an adenocarcinoma. Flexible endoscopy of the rectum was carried out and showed no abnormalities. Upper gastrointestinal endoscopy showed a stenotic tumour below the level of the papilla, displaying high-grade dysplasia and intramucosal carcinoma. Palliation was achieved with the placement of a self-expanding metal stent in the duodenum and palliative chemotherapy.

The patient's family history included three healthy brothers and one sister who had died from CRC at age 46 years. His mother had "polyps", but further data were not available.

MUTATION ANALYSIS

Germline

DNA analysis, using denaturing gradient gel electrophoresis analysis, including all coding exons of the *APC* gene, from both patients failed to detect a germline mutation. Additionally, in patient B, microsatellite analysis on tumour tissue showed a microsatellite stable phenotype. *MUTYH* gene sequence analysis identified a presumed homozygotic Tyr165Cys (c.494A→G) mis-sense mutation in patient A and Tyr165Cys (c.494A→G) and Pro391Leu (c.1172C→T) mutations in patient B, leading to the diagnosis of MAP. The *MUTYH* sequence analysis was carried out as described by Nielsen *et al.*⁶

Somatic

To provide further evidence that the duodenal tumours were caused by *MUTYH* deficiency rather than being a sporadic lesion, we screened for somatic mutations in codons 12 and 13 of the *K-RAS2* gene and codons 1286–1513 of the mutation cluster region of the *APC* gene in DNA extracted from the paraffin wax-embedded tumour samples. In both cases, *MUTYH*-associated GGT→TGT (Gly12Cys) mutations in codon 12 of the *K-RAS2* gene were identified in duodenal tissue⁷ (table 1). *K-RAS2* mutation analysis of two colon

carcinomas and colon adenomas (caecum and colon ascendens) of patient A and the sigmoid carcinoma of patient B also showed GGT→TGT mutations in codon 12. In two adenomas non-MUTYH-associated GGC→GAC transitions in codon 13 of the *K-RAS2* were detected. Protein-truncating *APC* mutations were not identified; however, sequence analysis in both duodenal and sigmoid carcinomas suggested loss of heterozygosity, which could be analysed owing to the presence of two polymorphisms in the *APC* DNA sequence.

DISCUSSION

Adenocarcinomas of the small bowel are relatively uncommon, accounting for approximately 2.4% of all gastrointestinal tumours.⁸ About 55% of small bowel cancers arise in the duodenum.⁹ In patients with FAP, a cumulative incidence of duodenal adenomas of 90% is found and about 5% develop duodenal carcinomas.¹⁰ Likewise, in patients with AFAP, duodenal adenomas are often observed and duodenal cancer has been described in a few patients¹¹ and in one patient as the presenting manifestation.¹² Among 157 patients with MAP reported so far, there were only eight patients with duodenal neoplasms.^{2-6 13} As endoscopy of the upper gastrointestinal tract is not routinely carried out in patients with between 10 and 100 polyps, the identified 5% (8/157) is most likely to be an underestimation. Here we described two patients with duodenal carcinomas and MAP.

The identification of somatic G→T mutations in codon 12 of the *K-RAS2* gene provides evidence that the duodenal lesions were caused by MUTYH deficiency rather than being a sporadic lesion. We could not detect any protein-truncating *APC* mutations as were initially described in most colorectal MAP lesions.¹ However, our observation is compatible with more recent and larger series where protein-truncating *APC* mutations, induced by G→T transversions, were found in only 21% (22/105) of adenomas and in 43% (6/14) of carcinomas.⁷ Alternatively, carcinoma of the small intestine may follow a different genetic pathway than colorectal carcinoma, as has been suggested recently by Wheeler *et al.*¹⁴

More studies are needed to identify the risk of developing duodenal adenomas and carcinoma, and to provide evidence-based recommendations for screening in patients with MAP. In the mean time, upper gastrointestinal screening with

careful inspection of the papilla for patients with MAP seems justified. A schedule comparable with FAP seems reasonable until further studies are available: starting from age 25–30 years, with 1–5 year intervals depending on findings.

METHODS

DNA isolation

Genomic DNA from four carcinoma and 13 adenoma tissues was extracted from paraffin wax-embedded material as described previously by de Jong *et al.*¹⁵

K-RAS2 mutation screening

Four carcinomas and 13 adenomas were screened for mutations in codons 12 and 13 of *K-RAS2*, by sequencing analysis. PCR was based on a protocol previously reported by Brink *et al.*¹⁶ A flanking 179 bp PCR product was amplified (annealing temperature 58°C) using the primers 5'-AGG CCT GCT GAA AAT GAC TGA ATA-3' (sense primer) and 5'-CTG TAT CAA AGA ATG GTC CTG CAC-3' (antisense primer). The resulting fragment was used as a template to amplify a 114 bp fragment including codons 12 and 13 using the primers 5'-AAA ATG ACT GAA TAT AAA CTT GTG G-3' (sense primer) and 5'-CTC TAT TGT TGG ATC ATA TTC GTC-3' (antisense primer), annealing temperature 50°C. Primer 5'-AAA ATG ACT GAA TAT AAA CTT GTG G-3' was chosen for sequence analysis (carried out at Base Clear LABSERVICES, Leiden, the Netherlands) of the Montège PCRμ96 plate (Millipore, Billerica, Massachusetts, USA) purified PCR products. Data were analysed with Chromas 1.5.

APC mutation cluster region mutation screening

Two carcinomas and three adenomas were tested for the presence of mutations in the mutation cluster region of *APC*. PCR was carried out with primer sets: S1 (173 bp), S2 (211 bp), S3 (214 bp) and S4 (206 bp) previously reported by Luchtenborg *et al.*¹⁷ PCR products were purified with Montège PCRμ96 plate (Millipore). Sequencing analysis of PCR products was carried out at the Leiden Genome Analysis Centre. Sequencing reactions were run on an ABI3700 (Applied Biosystems, Foster City, California USA) and analysed with chromas 1.5.¹⁷

Table 1 Somatic mutations in tumour material derived from patients A and B

Tumour	<i>K-RAS2</i> codon 12 (amino acid change)	<i>K-RAS2</i> codon 13 (amino acid change)	<i>APC</i> MCR (amino acid change)
Patient A			
Duodenal carcinoma	GGT→TGT (G12C)	wt	4442 GTT→GAT (V1481N) 4479 ACG→ACA (T1493T) 4539 GAG→GAA (E1513E)
Caecum carcinoma	GGT→TGT (G12C)	wt	ND
Colon ascendens carcinoma	GGT→TGT (G12C)	wt	ND
Colon adenoma	GGT→TGT (G12C)	wt	ND
Colon adenoma	GGT→TGT (G12C)	wt	ND
Colon adenoma	Wt	wt	ND
Colon adenoma	GGT→TGT (G12C)	wt	ND
Colon adenoma	GGT→TGT (G12C)	wt	ND
Colon adenoma	GGT→TGT (G12C)	wt	ND
Colon adenoma	GGT→TGT (G12C)	wt	ND
Colon adenoma	Wt	GGC→GAC (G13D)	4479 ACG→ACA (T1493T)
Colon adenoma	Wt	wt	ND
Colon adenoma	GGT→TGT (G12C)	wt	ND
Colon adenoma	Wt	GGC→GAC (G13D)	4479 ACG→ACA (T1493T)
Colon adenoma	GGT→TGT (G12C)	wt	ND
Patient B			
Duodenal adenoma*	GGT→TGT (G12C)	wt	4479 ACG→ACA (T1493T)
Sigmoid carcinoma	GGT→TGT (G12C)	wt	4479 ACG→ACA (T1493T)

E1513E, Glu1513Glu; G12C, Gly12Cys; G13D, Gly13Asp; MCR, mutation cluster region; ND, not done; T1493T, Thr1493Thr; V1481N, Val1481Asp; wt, wild type.

*In patient B, no tissue from the duodenal carcinoma was available for analysis, as this patient was treated conservatively.

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This study was funded by the Dutch Digestive Diseases Foundation, grant MWO 0355 (<http://jcp.bmjjournals.com/misc/ifora/licence-form.shtml>).

Competing interests: None.

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Accepted for publication 24 August 2005

Published Online First 30 August 2006

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