LETTER TO JMG

Genetic characterisation of patients with multiple colonic polyps

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Two main hereditary colorectal cancer syndromes have been described, namely familial adenomatous polyposis coli (FAP) and hereditary non-polyposis colorectal cancer (HNPCC). FAP is a disease characterised by autosomal dominant inheritance where the affected subjects develop hundreds to thousands of adenomatous polyps throughout the whole colon, usually during their teenage years. This syndrome is also characterised by the development of a variable range of extracolonic manifestations.[1 2] Germline mutations in the *APC* gene are responsible for FAP. Almost all mutations are nonsense or frameshift and result in the premature truncation of the protein.[3–5] Patients with 5′ or 3′ mutations in the *APC* gene tend to present with fewer adenomas (0-100) and are described as having attenuated FAP (AAPC).[6 7] HNPCC is also characterised by dominant inheritance and by the development of colorectal as well as various extracolonic cancers. Some studies have now shown that colorectal cancers (CRCs) in HNPCC patients also derive from precursor lesions, although the progression from adenoma to carcinoma is probably a much faster process compared to the sporadic and FAP model.[8 9] HNPCC is caused by germline mutations in mismatch repair (MMR) genes, *MSH2*, *MLH1*, *PMS1*, *PMS2*, and *MSH6*. Accordingly, tumours originated through inactivation of the MMR pathway accumulate somatic mutations throughout the genome and especially in simple repeated sequences called microsatellites. Microsatellite instability (MSI) is the hallmark of most colorectal cancers (CRC) associated with HNPCC.

As previously reported,[10–12] there are a substantial number of patients presenting with an excess of colorectal tumours, both adenomas and carcinomas, although they do not fulfil the diagnostic criteria for either FAP or HNPCC. These subjects usually present with fewer than 100 polyps, sometimes associated with synchronous or metachronic carcinomas, without accompanying extracolonic features and often with a poorly described family history, if any. The genetic basis for these syndromes is still poorly understood and inconsistent findings have been reported. Pedemonte *et al*[11] found that 5/18 patients with synchronous colorectal adenomas carried novel germline *APC* variants, whereas Beck *et al*[10] found one germline missense mutation in the *MLH1* gene (I219V) of uncertain functional effect in a cohort of 25 patients with similar clinical manifestations. Frayling *et al*[12] found that two missense substitutions in the *APC* gene, I1307K and E1317Q, were associated with the presence of multiple colorectal adenomas or carcinomas in a minority of a larger cohort. More recently, Tomlinson *et al*[13] have provided evidence based on linkage analysis for a new colorectal cancer gene, *CRAC1*, mapping to chromosome 15q14-q22 in an Ashkenazi family with a dominantly inherited predisposition to colorectal adenomas and carcinomas.[13] However, a systematic analysis of the suppressor and mutator pathways in this type of patient has not been carried out yet.

The aim of the present study was to evaluate in a group of patients with a marked excess of colorectal adenomas, often associated with carcinomas, whether a genetic defect could be detected in one of the two major pathways responsible for colorectal tumorigenesis, namely the WNT transduction pathway and DNA mismatch repair. We have analysed microsatellite instability as well as immunoexpression of MMR proteins (MSH2, MLH1, and MSH6) and of β-catenin. Germline mutations in *APC* or in the MMR genes have been investigated in those cases where the MSI and/or IHC analyses were suggestive of a defect in either WNT signal transduction or mismatch repair pathways.

MATERIAL AND METHODS

Patient selection

The study was approved by the local ethics and scientific committees and informed written consent was obtained from living patients entering the study.

Sixteen patients with a number of adenomas varying between six and 50 were included in this study; 8/16 patients also presented with synchronous or metachronic colorectal carcinomas as shown in table 1. Histopathological characteristics were specifically evaluated by two independent pathologists. For genetic analysis, tumour tissue was microdissected from 26 adenomas and eight carcinomas belonging to these 16 patients.

Microsatellite instability/LOH analysis

For each sample (adenoma or carcinoma), 10 sections of 10 µm each were cut parallel to the section used for histological classification and the DNA was extracted from paraffin embedded tissues using a proteinase-K digestion method followed by phenol-chloroform extraction. Constitutional DNA was isolated from peripheral blood using a salting out procedure.[14]

DNA was PCR amplified at different loci (D2S123, D5S346, BAT-25, BAT-26, and TP53). Briefly, DNA was amplified by PCR with the incorporation of [^{32}P]-αdCTP. PCR products were diluted with a denaturing buffer (95% formamide, 0.01% xylene cyanol, 0.01% bromophenol blue, 20 mmol/l NaOH) and subsequently denatured and electrophoresed on polyacrylamide gels containing 6.9 mol/l urea and 32.5% formamide. Gels were exposed overnight to MP film (Amersham Corp, UK) at −70°C.

Microsatellite instability was defined as the presence of novel alleles observed in neoplastic DNA but not present in the corresponding normal DNA.

Abbreviations: FAP, familial adenomatous polyposis coli; HNPCC, hereditary non-polyposis colorectal cancer; AAPC, attenuated FAP; MMR, mismatch repair; MSI, microsatellite instability; CRC, colorectal cancer; PTT, protein truncation test; DGGE, denaturing gradient gel electrophoresis

Table 1 Clinical characterisation of patients included in the study and clinical and pathological characterisation of carcinomas and adenomas

Patient	Age at diagnosis (y)	Familial history (CRC)	No of carcinomas	No of adenomas	Localisation of adenomas	Size of adenomas (cm)	Histological characterisation of the adenomas
1	75	No	1 (right colon)	20–50	Whole colon	<2	TA (LGD and HGD)
2	70	No	1	29	Whole colon	0.2–2	TA (LGD) and hyperplastic polyps
3	66	No	2 (right colon)	6	Whole colon	–	TA and TVA (LGD and HGD)
4	70	No	0	12	Whole colon	–	TA and TVA (LGD and HGD)
5	44	No	1 (right colon)	50	Whole colon	0.5–1	TA (LGD)
6	35	No	0	20–30	Whole colon	0.5–3	TA and TVA (LGD)
7	73	No	0	30	Whole colon	1–4	TA and TVA (LGD and HGD)
8	42	No	0	40–50	Whole colon	0.2–2	TA and TVA (LGD) and hyperplastic polyps
9	57	No	0	28	Whole colon	0.5–2	TA and TVA (LGD and HGD) and hyperplastic polyps (n=1)
10	48	Yes	1 (rectum)	30–40	Whole colon	<1	TA (LGD)
11	69	No	1 (right colon)	43	Whole colon	0.5–2	TA and TV (LGD) and hyperplastic polyps (n=37)
12	73	No	1 (right colon)	30	Whole colon	–	–
13	56	No	1 (left colon) and 2 (right colon)	8	Whole colon	0.2–1.4	TA and TVA (LGD)
14	73	Yes	0	13	Right colon	0.3–2	TA (LGD), hyperplastic (n=2) and serrated polyps (n=1)
15	34	Yes	0	7	Whole colon	0.4–2	TA and TVA (LGD and HGD) and hyperplastic polyps (n=1)
16	70	Yes	1 (left colon, mucinous)	9	Left colon	0.2–3	TA and TVA (LGD) and hyperplastic polyps (n=1)

TA, tubular adenoma; TVA, tubulovillous adenoma; LGD, low grade dysplasia; HGD, high grade dysplasia.

Mutational analysis

For *APC* mutation analysis of exon 15, the protein truncation test (PTT) was used as described by Powell *et al*.[5] Exon 15 was divided into four overlapping fragments that were PCR amplified. The 5′ primers used for amplification of these fragments have in their 5′ end a T7 promoter sequence used for transcription initiation as well as a sequence for translation onset. In vitro translated proteins were then separated on a 14% gradient SDS-polyacrylamide gel and visualised after autoradiography. Exons 1-14 of the *APC* gene were analysed using denaturing gradient gel electrophoresis (DGGE) with primers previously described,[15 16] with one of the primers containing at its 5′ end a GC rich sequence (GC clamp) to increase screening efficiency. The amplified product was then loaded on a 6% polyacrylamide gel containing a linearly increasing denaturant gradient (100% denaturant=7 mol/l and 40% formamide (v/v), acrylamide:bisacrylamide=39:1). The electrophoresis was performed at 160 V in TAE buffer (40 mmol/l Tris-acetate pH 7.5, 20 mmol/l sodium acetate, 1 mmol/l Na_2 EDTA) at a constant temperature of 60°C for four hours. Finally, the gel was stained with ethidium bromide and visualised under UV light. DGGE was also used to analyse fragment G from exon 15 (codons 1263-1377) using previously described primers.[17]

Mutation analysis in MMR genes was performed using DGGE with previously described primers.[18 19]

Whenever there was a pattern corresponding to the presence of homo- or heteroduplexes for a specific exon on DGGE or a truncated protein on PTT, we then proceeded to sequence with the same primers but without the GC rich sequence, and using the f-mol sequencing kit (Promega Corporation, USA).

Immunoexpression analysis

MSH2, MLH1, MSH6, and β-catenin expression was analysed using immunohistochemistry as previously described.[20] In brief, paraffin embedded sections (5 μm) were prepared, deparaffinised with xylene, and rehydrated in graded alcohols and phosphate buffered saline (PBS). The avidin-biotin-peroxidase complex technique was used for immunohistochemical staining with diaminobenzidine/0.02% hydrogen peroxide development. Counterstaining was performed with Meyer's haematoxylin. For MSH2, MLH1, and MSH6 proteins, a positive reaction was recognised whenever there was unequivocal nuclear staining in the tumour cells. Tumour cells without nuclear staining, in the presence of normally stained non-neoplastic stromal cells, were considered to exhibit an abnormal pattern of expression. β-catenin expression was evaluated by the transition from membrane expression, occurring in normal cells, to nuclear expression. The nuclear expression was scored as –, +, ++, and +++, according to the percentage of cells expressing β-catenin in the nucleus: absence of nuclear expression (–), less than 25% (+), 25-50% (++), and more than 50% (+++).

RESULTS

The clinicopathological and family history data of the patients included in the present study are summarised in table 1. The cohort included 10 males and 6 females and the mean age at diagnosis was 60. The patients' age at diagnosis ranged from 34 to 75 years. The median number of colonic adenomas was 25 (range six to 50). None of the patients had features of classical FAP. However, there were also eight patients who presented with 11 colorectal carcinomas, most of them located in the right colon (8/11 tumours), a few of them arising in the fourth decade of life, and others associated with a lower number of colonic adenomas. Thus, although the family history was negative in all but four patients, the study group presented with clinical features that could be suggestive of attenuated or atypical FAP or HNPCC.

Histological characterisation of colonic polyps showed that most lesions analysed were tubular or tubulovillous adenomas, except for patient 11 where the large majority of polyps were hyperplastic lesions (table 1).

Table 2 summarises the results obtained for the colonic tumours analysed. In most cases two or more specimens were analysed per patient. Whenever a carcinoma was present it was always included in the analysis. With respect to MSI analysis, all the carcinomas and adenomas examined were scored as MSS. Use of these markers also showed that 7/16 (44%) patients showed LOH or allelic imbalance at several loci (D2S123, D5S346, and TP53) (table 2). This was observed in 4/8 carcinomas and in 8/25 adenomas analysed. Fig 1 shows LOH for D2S123 and D5S346 in the carcinoma from patient 2. To confirm that most of these lesions did not arise from MMR mutations, expression of MSH2, MLH1, and MSH6 was evaluated by immunohistochemical analysis of all the available tumours. Normal expression patterns were observed in all

Table 2 Results of microsatellite instability/LOH analysis and *MSH2*, *MLH1*, *MSH6*, and β-catenin protein immunoexpression for carcinomas and adenomas from patients included in the study

Patient	No of adenomas	Analysed samples	MSI analysis	LOH analysis	Immunoexpression			
					MSH2	MLH1	MSH6	Nuclear β-catenin
1	20–50	Carc	MSS	LOH (TP53)	Y	Y	Y	++
		Ad 1 (LGD)	MSS	N	Y	Y	Y	+
		Ad 2 (LGD, HGD)	MSS	N	Y	Y	Y	++ (LGD), +++ (HGD)
2	29	Carc	MSS	LOH (D2S, D5S,TP53)	Y	Y	Y	++
		Ad (LGD)	MSS	LOH (D2S), AI (D5S)	Y	Y	Y	+++
3	6	Carc	MSS	N	Y	Y	Y	+
		Ad (HGD)	MSS	N	Y	Y	Y	+++
4	12	Ad (LGD, HGD)	MSS	LOH (D2S), AI (D5S)	Y	Y	Y	+++
		Ad (LGD)	MSS	AI (D2S, D5S)	Y	Y	Y	+++
5	50	Carc	MSS	LOH (D2S)	No	Y	No	–
		Ad (LGD)	MSS	LOH (D2S)	Y	Y	Y	+
6	20–30	Ad 1 (LGD)	MSS	N	Y	Y	Y	++
		Ad 2 (LGD)	MSS	N	Y	Y	Y	+
		Ad 3 (LGD)	MSS	N	Y	Y	Y	+++
7	30	Ad 1 (LGD)	MSS	N	Y	Y	Y	–
		Ad 2 (LGD)	MSS	N	Y	Y	Y	+++
8	40–50	Ad (LGD)	MSS	N	Y	Y	Y	+
9	28	Ad 1 (LGD)	MSS	AI (D5S)	Y	Y	Y	+
		Ad 2 (LGD)	MSS	N	Y	Y	Y	++
10	30	Carc	MSS	N	Y	Y	Y	+++
		Ad 1 (LGD)	MSS	N	Y	Y	Y	–
		Ad 2 (LGD)	MSS	N	Y	Y	Y	+++
11	40	Carc	MSS	N	Y	Y	Y	+++
		Ad 1 (HGD)	MSS	N	Y	Y	Y	+++
		Ad 2 (LGD)	MSS	N	Y	Y	Y	+++
12	30	Carc	MSS	LOH (D2S), AI (TP53)	Y	Y	Y	+
13	8	Carc	MSS	N	Y	Y	No	Rare
		Ad 1 (LGD)	MSS	N	Y	Y	Y	–
		Ad 2 (LGD)	MSS	N	Y	Y	Y	–
14	13	Ad (LGD)	MSS	N	Y	Y	Y	–
15	7	Ad 1 (LGD)	MSS	LOH (D5S), AI (D2S)	Y	Y	Y	+++
		Ad 2 (LGD)	MSS	AI (D5S)	Y	Y	Y	+++
		Ad 3 (LGD)	MSS	LOH (D5S)	Y	Y	Y	++
16	9	Ad 1 (LGD)	MSS	N	Y	Y	Y	–

MSI, microsatellite instability; MSS, microsatellite stability; LOH, loss of heterozygosity; AI, allelic imbalance; D2S, D2S123; D5S, D5S346; N, normal; Y, yes (normal expression); nuclear expression of β-catenin (– absence of expression; + less than 25% expression; ++ between 25% and 50% expression; +++ more than 50% expression).

cases with the exception of the carcinomas from patients 5 and 13 where no MSH2 and MSH6 expression was detected. The adenomas analysed for these patients showed a normal expression of these proteins, thus there is no indication that the adenomas show any defect in the MMR genes. In order to evaluate the presence of any possible germline defect, germline mutations in the corresponding MMR genes were analysed in these patients. A missense mutation (GGC→GAC) was found in codon 322 of *MSH2* exon 6 in patient 5. This results in substitution of the amino acid glycine for aspartate. Germline variants at this codon have been detected in HNPCC patients but also in apparently normal subjects.[21 22] MSI was not observed at any of the markers analysed in the corresponding carcinoma, though MSI-H is only observed in 95% of HNPCC associated tumours. The absence of MSI was further evaluated by using the markers BAT-40 and *MYCL* that are more specific for analysing the presence of MSI-L.

Since the majority of the lesions analysed in this series seemed to follow the suppressor pathway (frequent LOH, absence of MSI, and normal expression of MMR proteins in the great majority of tumours), we set out to search for germline mutations in the entire coding region of the *APC* gene in the 16 patients. No mutations were detected in any of the patients included in the study. Because PTT does not detect I1307K and E1317Q variants, codons 1263-1377 were analysed by DGGE in each of the patients but again no mutations were found (data not shown).

As shown in table 2, nuclear expression of β-catenin was detected in the vast majority of tumours analysed: in 20/26 adenomas and 7/8 carcinomas. Fig 2B shows an example of membrane and nuclear β-catenin expression in one carcinoma. Although the numbers are clearly too small, we observed a tendency for higher β-catenin expression in adenomas with a high grade dysplasia as compared to

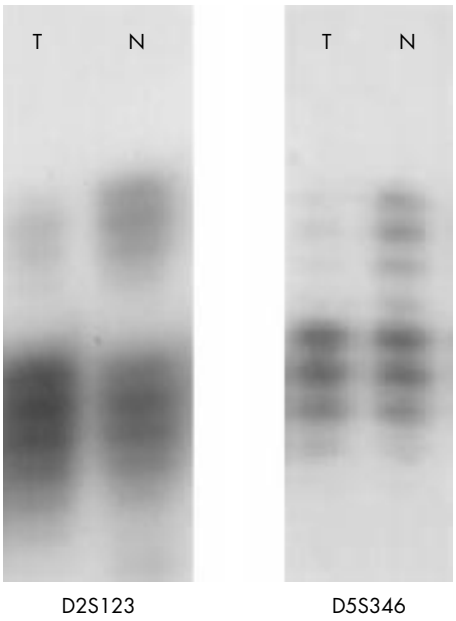

Figure 1 Presence of LOH for microsatellite markers D2S123 and D5S346 in the carcinoma from patient 2. T=tumour; N=normal colonic mucosa.

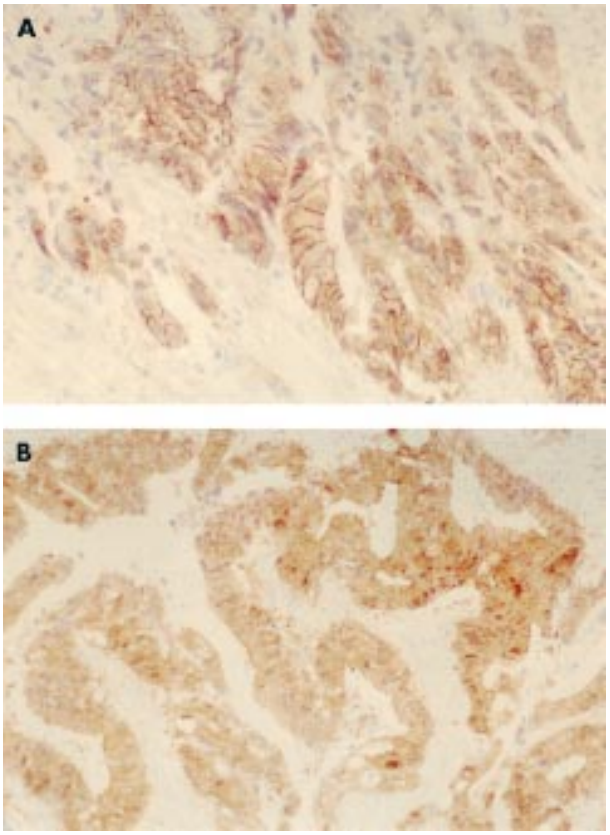

Figure 2 β-catenin immunoexpression. (A) Carcinoma cells without expression of β-catenin in the nucleus. (B) Carcinoma cells showing expression of β-catenin in the nucleus.

carcinomatous areas (4/4 *v* 2/8, p=0,014), which was statistically significant.

DISCUSSION

There is increasing evidence that some patients with colorectal tumours have features of both FAP and HNPCC.[10–12] A few published studies have searched for potential germline defects in CRC predisposing genes. Results published so far are conflicting, because while some studies reported germline variants of the *APC* gene that could predispose to the development of multiple colorectal adenomas and carcinoma,[11 12] others could not find mutations in this gene.[10] Moreover, germline HNPCC mutations of uncertain functional effect were found in a minority of the patients analysed by Beck *et al*.[10] Most patients included in the present series had a clear excess of colorectal adenomas, which could suggest an *APC* related phenotype. However, a substantial number of them also presented with colorectal cancers predominantly located in the right colon, suggestive of a HNPCC-like syndrome.

Notably, the majority of these patients do not have a family history of colorectal cancer. However, the increased tumour multiplicity is highly suggestive of a hereditary cancer syndrome. The absence of family history could be explained either by de novo germline events or, alternatively, by low penetrance variants.

As clinical expression of the patients included in this study could not be clearly classified as FAP or HNPCC, we used MSI, LOH, and IHC analysis of tumour samples to establish whether the tumour exhibited either microsatellite or chromosomal instability. All tumours of the present series were MSS and immunoexpression of MMR proteins was normal for all three proteins analysed, except for two patients. In patient 5, MSH2 and MSH6 expression was negative in the carcinoma specimen and a germline missense mutation in exon 6 of *MSH2* gene was found. This variant has been described both as a pathogenic mutation as well as a rare polymorphism.[21 22] Although absence of MSI-H or MSI-L in colorectal tumours has been observed in patients harbouring pathogenic mutations in *MSH6* or *MLH1*,[23 24] this has not been described for patients carrying mutations in the *MSH2* gene. Patient 5 clearly exhibited a colonic phenotype, which could easily be classified as an atypical polyposis, with 50 colonic adenomas distributed throughout the whole colon and a carcinoma of the splenic flexure at the age of 44. However, this was the only case of carcinoma negative for nuclear expression of β-catenin, which is rarely observed in tumours arising in the context of a germline defect of the *APC* gene.[25] Interestingly, LOH was observed in the wild type allele at the D2S123 locus where the *MSH2* gene is located. Because there is no family history, segregation analysis was not possible and functional studies of the MSH2 protein may be required in this case for further clarification. MSH6 mutation analysis will also be important to characterise this case further.

A tumour sample from patient 13 also showed absence of MSH6 expression. Despite no germline mutations being found in the MSH2 and MLH1 genes of this patient, we cannot exclude the involvement of MMR genes. Also supporting a possible involvement of the mutator pathway in this case is the lower number of adenomas compared with most patients included in this study, the absence of LOH, and the rare or absent nuclear β-catenin expression in the carcinoma and adenomas, respectively.

In the majority of tumours analysed in the present study, evidence was found for LOH and allelic imbalances at several loci. Therefore, germline mutations in the *APC* gene might be responsible for the florid phenotype observed in most of our patients. However, and in contrast with previous reports,[11 12] we could not find any *APC* mutations. Pedemonte *et al*[11] found that five out of 18 patients with an excess of adenomas carried novel germline *APC* variants. However, as they stated, the pathogenicity of these variants is still uncertain though most of the patients had a clear cut family history of CRC. Also, Frayling *et al*[12] studied a set of 164 patients with multiple colorectal adenomas and/or carcinomas and found that three patients, all of whom were of Ashkenazi descent, carried the I1307K variant, while four other patients had a germline E1317Q missense variant. None of these variants was found in

the control group. Recently, Lamlum *et al*[26] screened 164 unrelated patients with multiple colorectal adenomas and found that the E1317Q missense variant accounted for approximately 4% of these patients. In this study, truncating *APC* variants in exon 9 and in the 3′ of the gene were also found in 4/164 patients, all of them with a family history.

Considering the large number of patients included in the latter studies, it is not surprising that we did not find any of these missense variants or truncating mutations (5′ to exon 5, exon 9, and 3′ to codon 1580) in the admittedly limited number of patients investigated here. Our results are similar to those of Beck *et al*[10] where no *APC* variants were detected in a group of 25 patients who had an unusually large number of colorectal adenomas and a missense variant of a MMR gene.

In the present series, we also found that a large proportion of adenomas and carcinomas analysed showed abnormal β-catenin expression. β-catenin expression was observed in the nucleus and not in the membrane as in normal tissue, thus supporting the involvement of the Wnt pathway. A number of germline defects could result in the nuclear localisation of β-*catenin* in most neoplastic lesions analysed here. As mutations in β-*catenin* are usually dominant,[27 28] it does not seem likely that germline oncogenic alterations in β-*catenin* would predispose to this mild phenotype. Alterations in other genes such as *conductin*, *axin*, and *GSK-3*β, previously implicated in the control of β-catenin signalling to the nucleus,[29–32] might also be responsible for our IHC findings. Although mutations in these genes have not been found in hereditary colorectal cancer patients,[33] subjects with an excess of colorectal adenomas may represent a different category altogether where these genes have not been analysed yet.

Also, from the present analysis we cannot exclude the presence of mutations in the *APC* promoter or other regulatory regions of this gene, which could affect *APC* expression, thereby interfering with β-catenin degradation and increasing its nuclear expression. Mutations in regulatory or non-coding regions of the *APC* gene are difficult to detect by standard mutational analysis.[34]

In the present study, we also found that nuclear expression of β-catenin was more pronounced in adenomas with high grade dysplasia as compared to invasive carcinomas. This is similar to what has been described by Samowitz *et al*,[35] where the percentage of somatic β-*catenin* mutations was significantly higher in small adenomas as compared to larger ones and invasive cancers. As these authors suggest, this might indicate that β-*catenin* mutations may be especially advantageous in the early stages of colorectal carcinogenesis, or that β-*catenin* mutations could reduce the likelihood of adenoma progression.

In conclusion, the occurrence of frequent LOH and allelic imbalances in most of the tumours included in the present series suggests that the suppressor pathway is involved in the development of multiple colorectal adenomas. The presence of nuclear expression of β-catenin in the great majority of neoplasms analysed suggests the involvement of the Wnt transduction pathway during tumorigenesis in these cases.

Further work is necessary to provide evidence for new colorectal cancer genes associated with an inherited predisposition to multiple colorectal adenomas and carcinomas. Moreover, besides genetic factors, it cannot be excluded that environmental factors, such as diet, may also contribute, in some cases, to an increased number of adenomas.

However, the fact that, as is shown in this study, the mutator pathway does not seem to be involved in the development of this phenotype further reinforces that most probably these patients do not need to be analysed for germline mutations in the MMR genes.

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ECHO

Cancers of the small and large bowel originate differently

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Adenocarcinoma of the small intestine and colorectal cancer have different genetic pathways, suggests a molecular study from Oxford. Much rarer than colorectal cancers, cancers of the small intestine are difficult to diagnose and have a poor prognosis. They tend to be adenocarcinomas and are similar to colorectal cancers. So comparing the genetic pathways of each might indicate whether the small intestine is naturally resistant to cancer or might give some clues to clinically significant differences.

Using PCR and gene sequencing on DNA extracted from paraffin sections and immunohistochemical staining of sections with monoclonal antibodies, the researchers looked for particular genes and proteins in the progression to colorectal cancer in 21 non-familial, non-ampullary primary adenocarcinomas of the small intestine. These included mismatch repair genes hMLH1 and hMSH2; adenomatous polyposis coli (APC) gene mutations in the mutation cluster region; and proteins β-catenin, E-cadherin, and p53.

One cancer resulted from replication error. Significantly, no mutations were found in the mutation cluster region of the APC gene, but all cancers were positive for hMLH1 and hMSH2 repair genes. Expression of β-catenin and E-cadherin at the intercellular borders was reduced in 17 and eight of the cancers respectively, and p53 was overexpressed in the nuclei of five cancers.

Lack of mutations in the mutation cluster region of the APC gene in this relatively large study contrasts strongly with colorectal cancer, leading the researchers to conclude that the genetic pathways differ, even though aberrant expression of β-catenin, E- cadherin, and p53 are common features.

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