Figure 1 Methylation analysis of the APC promoter. (A) Map of the APC-1A promoter. The transcription start site of APC exon 1 (grey box) is at nucleotide +1. The position of the primers used to amplify a 229 bp fragment of the promoter upstream of exon 1 is shown by arrows. Individual CpG dinucleotides are denoted by vertical black bars. The diagnostic restriction enzyme sites that cleave specific CpG sites when methylated are indicated. (B) Gel showing an example of combined bisulfite and restriction analysis of APC-1A promoter. L, pUC19/MspI DNA ladder; C+, in vitro methylated genomic DNA shows cleavage of the methylated amplicons with RsaI to give a digestion pattern of 162 and 67 bp (and 141, 60, and 28 bp for TaqI, not shown); C-, unmethylated genomic DNA from a normal control individual remains undigested. Wells labelled 1-9 contain peripheral blood DNA from nine familial adenomatous polyposis subjects, each negative for APC-1A methylation.

carcinomas, but no methylation changes have been detected at the 1B promoter from which a minor transcript is derived, and so this latter promoter was not analysed.⁷⁻⁹ With the approval of the Human Research and Ethics Committee of St Vincent's Hospital, this study enrolled 140 individuals with colonic polyps from family cancer clinics in Australia and the UK. None of the individuals harboured pathogenic germline sequence mutations in the APC gene, and biallelic MYH mutations had been excluded in all but 10 cases. Of the 140 individuals, 22 were diagnosed with HPS (mean age 60.5 (9.6) years), 29 with FAP (mean age 27.8 (10.5) years), and 89 with attenuated FAP (mean age 59 (18.6) years).

Probands were screened for hypermethylation of the APC-1A promoter in peripheral blood DNA using the method of combined bisulfite and restriction analysis. Genomic DNA was converted by standard sodium bisulfite treatment and subject to polymerase chain reaction (PCR) amplification using primers 5'-GGG TTA GGG TTA GGT AGG TTG-3' and 5'-ACA CCT CCA TTC TAT CTC CAA TAA C-3', which are specific to bisulfite converted DNA, and co-amplify both methylated and unmethylated alleles with equal efficiency (fig 1A). PCR amplification was performed using FastStart Taq Polymerase (Roche Diagnostics, New South Wales, Australia) with a final concentration of 3 mM MgCl₂, and annealing at 55°C. The amplification products were digested separately with the restriction enzymes TaqI and RsaI, which specifically cleave amplicons derived from templates that are methylated at the corresponding CpG dinucleotides, whereas the unmethylated promoter remains undigested (fig 1). To serve as a positive control, genomic DNA was methylated in vitro using CpG M.SssI methylase (New England Biolabs, Ipswich, Massachusetts, USA). Genomic DNA from a normal control subject was used as a negative control.

No methylation of the APC-1A promoter was identified in the constitutional DNA of any of the probands studied, indicating germline epimutations of APC do not underlie the phenotype in these individuals. Our results suggest that germline epimutation of

APC is not a significant factor in the polyposis syndromes studied, and does not account for cases with no detectable sequence mutation of the APC or MYH genes. This study adds further support to the concept that germline changes in APC are not responsible for HPS. However, germline epimutations remain a possibility in polyposis cases where there is a marked allelic imbalance in APC transcription. Yan *et al* reported an FAP family in which there was considerably reduced expression of one specific allele in each of the family members carrying this haplotype.¹⁰ No sequence mutation within the APC gene, including the promoter and 3' untranslated region, was identified despite intensive screening. However, the methylation status of the APC-1A promoter was not investigated in this family. It remains possible that some families and isolated cases such as this may have germline epimutations of APC, causing the affected allele to be silenced.

Although somatic APC promoter methylation occurs in a spectrum of cancers, unlike the association of microsatellite instability with MLH1 methylation, it does not give rise to an obvious phenotype. Furthermore, while germline APC mutations are well known as causing multiple adenomatous polyps and increased cancer risk, it is likely that germline defects in other genes can produce a similar phenotype. In APC mutation negative cases that produce a florid syndrome in the absence of a strong family history, it may still be worth investigating epigenetic mechanisms of gene inactivation as well as genetic causes.

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Tablet induced dysphagia in oesophageal cancer

We report a case of an unusual pattern of dysphagia in an 82 year old man. He first presented in January 2005 with a six month history of dysphagia and heartburn.

Figure 1 Endoscopy of the oesophagus at 35 cm showing the tablet bolus.

Following the diagnosis of an adenocarcinoma of the oesophagus he declined surgery as well as chemo- and radiotherapy.

Three months later he was referred because of increasing dysphagia. He ate a normal breakfast, including toast, but found that his swallowing was poor for the rest of the day. By the following morning his symptoms had improved again.

On the morning of his clinic visit he had eaten his usual breakfast and then underwent an upper gastrointestinal endoscopy at midday. Endoscopy (fig 1) revealed a bolus in the oesophagus above the tumour. The bolus was removed with a dornier basket and a standard (11 mm) endoscope passed through the 3 cm long tumour. The patient saw the bolus and immediately identified it as the alfuzosin slow release tablet he was taking after breakfast.

Xatral XL tablets measure 8×6 mm and swell up to twice their original size in vivo. They slowly dissolve over 24 hours. For pharmacokinetic reasons patients are advised to take the medication after meals. Our patient was changed to a different medication for his benign prostate hyperplasia and did not experience this unusual pattern of dysphagia again.

Large tablets and capsules can cause dysphagia in patients suffering from conditions such as oesophageal tumours or achalasia. Slow release tablets may increase in size in vivo and often do not dissolve for hours. Therefore, when prescribing medications for

these patients, attention should be paid to choose the appropriate formulation of the drug.

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Risk of subsequent development of gastric cancer in patients with previous gastric epithelial neoplasia

The development of gastric epithelial neoplasia is closely linked to precursor conditions in the background mucosa.¹⁻³ Recently, endoscopic mucosal resection (EMR) has become widely used for the treatment of gastric neoplasia,⁴ resulting in almost complete conservation of the patient's stomach. To determine the risk of a second cancer in the stomach that once gave rise to epithelial neoplasia, we conducted a long term retrospective cohort study of 255 patients with primary gastric epithelial neoplasia who underwent curative resection by EMR between 1983 and 2002 at our hospital.

Characteristics of the subjects at the initial treatment are shown in table 1. Synchronous multiple neoplasias were confirmed in 19 (7.5%) of the 255 subjects; 56 patients had adenomas and 199 had cancer. In the eight patients with both gastric cancer and adenoma, cancer was taken as the representative histology. In this study, we defined lesions classified as category 3 in the Vienna classification⁵ as "adenoma" and categories 4 and 5 as "cancer".

A second gastric cancer developed in 43 of the 255 patients. The crude incidence rate of the second gastric cancer was 3.9 per 100 person years. Kaplan-Meier estimates showed that the three year and six year cumulative incidence rates of newly developed gastric cancers were 10% and 20%, respectively. The cumulative incidence of subsequent cancer increased steadily throughout the observation.

To compare the risk of new development of gastric cancer in our patients with that of general population in Japan,¹⁰ the standardised incidence ratio (SIR) was calculated. SIR for newly developed cancer in our patients was 10.4 (95% confidence interval 7.0-14.9). SIRs stratified according to sex, age, and synchronous multiplicity at the initial treatment are also shown in fig 1A.

Furthermore, we analysed the factors predicting subsequent gastric cancer development. Ten of the 56 adenoma and 33 of the 199 cancer patients had newly developed gastric cancer. The crude incidence rates of the second cancer were exactly the same (3.9 per 100 person years) in both groups, and the cumulative incidences did not differ between the two groups using the Kaplan-Meier method ($p = 0.97$; fig 1B). Patients with synchronous multiple lesions were at a significantly higher risk than those with a solitary lesion ($p < 0.01$; fig 1C). Multivariate analysis confirmed the above findings (table 1).

All of our patients were positive for *Helicobacter pylori* but did not receive eradication therapy because many underwent EMR before the mid 1990s. Thus a very high rate of *H pylori* infection appears to be at least partly responsible for the high incidence of the second cancer in our patients.⁶ However, the incidence in our patients was much higher than *H pylori* positive Japanese⁷ and Chinese⁸ patients without a history of gastric neoplasia. Thus factors other than *H pylori* infection were also involved in the development of the second gastric cancer in our patients with previous gastric neoplasia.

An important finding of our study was that patients with gastric adenoma had the same risk for the development of a subsequent gastric cancer as those with gastric cancer. In addition, we found that a considerable number of our patients with gastric adenoma (12.5%) had concurrent gastric cancer. Thus although gastric adenoma may rarely progress to cancer,⁹ it is likely that gastric cancers and adenomas develop in the same background of the gastric mucosa, probably with similar genetic alterations or environmental conditions.

Taken together, our results indicate that patients with a previous history of either gastric adenoma or cancer are at a higher risk of subsequent development of gastric cancer. Once neoplasia develops, the gastric mucosa

Table 1 Characteristics of the 255 patients at the initial endoscopic treatment and multivariate analysis* as predictors for subsequent gastric cancer

Characteristic		Hazard ratio	95% CI	p Value§
No of patients	255			
Male sex	183 (71.8%)	1.11	0.52-2.39	0.79
Age (years) (mean (SD))	67.9 (9.2)	1.54†	1.07-2.21	0.02
Histology				
Adenoma	56	0.98	0.48-2.00	0.95
Carcinoma†	199			
Synchronous multiplicity				
Solitary	236 (92.5%)			
Multiple	19 (7.5%)	4.10	1.92-8.79	<0.01
Adenomas	2			
Adenoma and carcinoma	8			
Carcinomas	9			
Surveillance period (months) (mean (SD))	51.6 (34.9)			

*The Cox proportional hazard model was used.

†Carcinoma was taken as the representative histology of the case when the patient had multiple neoplasias of both carcinoma and adenoma.

‡Increment of 10 years.

§p values were calculated using the Wald test.