

# PostScript

## LETTERS

### Plexiform neurofibroma mimicking a pancreatic cystic tumour

Pancreatic neurogenic tumours are extremely rare.<sup>1-3</sup> Among benign neurogenic tumours, schwannoma is more frequently encountered.<sup>2,3</sup> We report here the case of a plexiform neurofibroma, a type of neurogenic tumour in the pancreas, to our knowledge previously unreported.

#### History

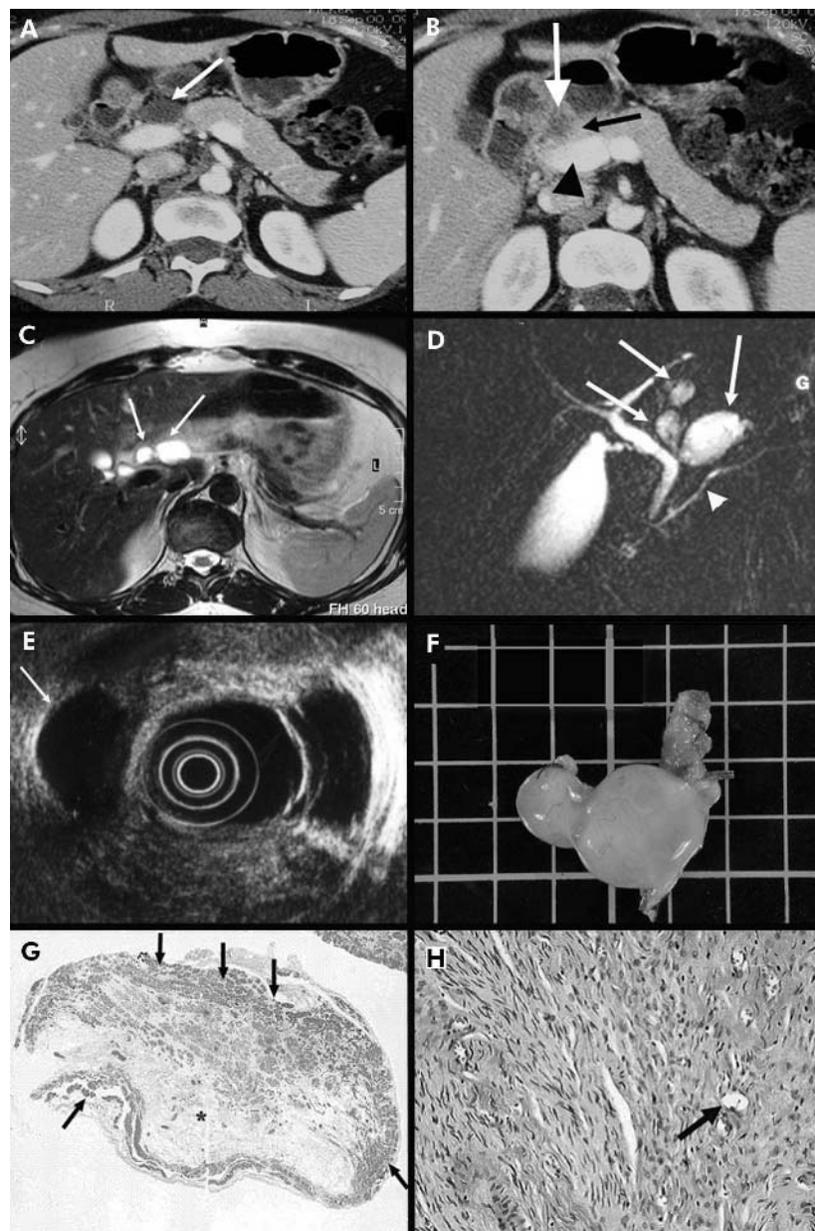
A 44 year old Caucasian female patient was hospitalised for epigastric and right abdominal pain lasting for seven months. Abdominal ultrasound and computed tomography showed a cystic lesion located in the superior and anterior part of the pancreatic isthmus, with a maximal diameter of 3.5 cm (fig 1A, B). T2 magnetic resonance imaging demonstrated a trilobar cystic lesion with strong hyperintensity (fig 1B); no communication with the main pancreatic duct was noted at magnetic resonance cholangiopancreatography (fig 1C). Endoscopic ultrasound (EUS) showed a cystic lesion containing heterogeneous fluid (fig 1D). EUS guided fine needle aspiration provided mucoid fluid with no epithelial cells. Fluid pancreatic enzyme concentrations were 423 and 1204 U/l for amylase and lipase, respectively, while CEA, CA 19.9, and CA 72.4 were 17 ng/ml, 9 U/ml, and 140 U/ml, respectively. Despite the low CA 19.9 concentration and lack of mucinous cells in cystic fluid, other findings were consistent with a diagnosis of mucinous cystadenoma. Surgical exploration confirmed a cystic lesion of the superior part of the pancreatic isthmus, distant from the main pancreatic duct (fig 1A, B). Tumour enucleation was performed. On macroscopy there was a well delineated, trilobated, translucent mass, measuring 3.5 cm (fig 1E). The tumour consisted of aggregates of benign spindle cells embedded in a fibrillar matrix (fig 1F). These aggregates formed a thin rim around a large central low cellular zone of oedema and myxoid degeneration. The tumour cells expressed neurofilaments and S100 protein on immunohistochemistry. P53 immunostaining was negative and sparse nuclei were Ki67 positive. These features were consistent with a benign plexiform neurofibroma (PNF). No neurofibromatosis related lesions were found and no mutation of the *NF1* (neurofibromatosis 1) gene was identified on analysis of DNA both from blood lymphocytes and tumour tissue. At follow up, two years after surgical resection, the patient did not present with any complaints and there was no evidence of pancreatic lesions.

#### Discussion

The presence of PNF in the pancreas has several clinical implications, as indicated by the present case. Firstly, PNF may mimic a pancreatic cyst, as was hypothesised in this case before surgery. The cystic appearance of

neurogenic tumours is frequently encountered, with intratumoral oedematous and myxoid changes probably being the under-

lying lesions.<sup>4</sup> A bright appearance on T2 weighted magnetic resonance images is a characteristic of PNF.<sup>5</sup> Secondly, surgical



**Figure 1** (A) Enhanced computed tomography. Hypoattenuating round lesion located in the anterior part of the pancreatic isthmus (arrow). (B) The caudal part of the main lesion is encased in the isthmus of the pancreas (white arrow). The pancreatic parenchyma is normal upstream (black arrow). The main portal vein is normal, distant from the lesion (arrowhead). (C) T2 magnetic resonance imaging. The lesion is strongly hyperintense as cysts; another similar lesion was seen on the right side (arrows). (D) Magnetic resonance cholangiopancreatography with thick slice. The two lesions are well visible, with a third one indicated (arrows). The main pancreatic duct is normal (arrowhead) with no obvious communication with the lesions. (E) Endoscopic ultrasound. Anechoic cystic lesion without defined cyst wall or mural nodule (arrow). (F) Surgical specimen consisted of a bilobated, firm, translucent, well delineated mass. (G) On microscopy, at low magnification, the lesion was heterogeneous with a solid cellular part (arrows) and a central oedematous acellular zone (\*), giving the pseudocystic aspect of the lesion (haematoxylin and eosin stain, magnification  $\times 10$ ). (H) At high magnification, the solid part of the lesion was composed of a regular spindle cell proliferation. Intratumoral vessels showed a thin fine wall (arrow) (magnification  $\times 40$ ).

resection was necessary to exclude malignancy which is more frequently encountered in PNF compared with classical neurofibromas.<sup>2</sup> In addition to classical benign features, similar to published data on benign PNF,<sup>6,7</sup> a high cell proliferation and p53 protein expression were absent in our case. Thirdly, PNF is a morphological variant of neurofibroma, generally considered pathognomic for an NF1 syndrome.<sup>8</sup> When diagnosed in adult patients, it is frequently a solitary tumour and is considered a mosaic located form of NF1 syndrome.<sup>9</sup> The absence of detectable genetic abnormalities and other clinical NF1 syndrome associated lesions in the present case could be explained by such a mechanism.<sup>9</sup> For these patients, there is a low risk of developing other diseases associated with NF1 syndrome.

In conclusion, we have reported an uncommon case of PNF, unique in its pancreatic location. Intratumoral myxoid and oedematous changes that develop in this type of neurofibroma give a cystic appearance which may lead to a misdiagnosis of a pancreatic cyst. Such lesions should be added to the list of benign pancreatic tumours with a cystic appearance.

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## No genetic association between EPHX1 and Crohn's disease

In a case control study on the associations between functional genetic polymorphisms in biotransformation enzymes and Crohn's disease, we found a strong association between the Tyr113His (348T>C) polymorphism in exon 3 of the microsomal epoxide hydrolase (EPHX1) gene and Crohn's disease.<sup>1</sup> The three referees all agreed that the study was interesting and should be published so that other groups can attempt to replicate the results in independent study cohorts. This was done recently by Cuthbert and colleagues (*Gut* 2004;**53**:1386) who investigated 344 controls and 307 patients with Crohn's disease, and who were unable to reproduce our results. In addition, they reported that our data for the EPHX1 exon 3 polymorphism in the control group were not in Hardy-Weinberg equilibrium (HWE), as also noticed previously by Györfy and colleagues.<sup>2</sup> Our data on EPHX1 exon 3 genotyping were obtained by restricted fragment length polymorphism (RFLP) analyses by applying the method described by Lancaster and colleagues.<sup>3</sup>

However, recently it was reported that a silent substitution polymorphism (G to A) at codon 119 of the EPHX1 gene may exist, which may flaw the polymerase chain reaction (PCR) RFLP method applied by us, as the presence of this polymorphism may disturb proper binding of the reverse primer, covering the 119 G>A area, resulting in over-classification of His113 alleles.<sup>4,5</sup> Therefore, we developed a dual colour allele specific discrimination assay for genotyping the polymorphism at codon 113 of the EPHX1 gene. EPHX1 genotypes were detected with the iCycler iQ Multicolour Real Time Detection System (Bio-Rad Laboratories, Veenendaal, the Netherlands) using molecular beacons. PCR was performed with the forward primer 5'-CAA CTC CAA CTA CCT GAA G-3' and the reverse primer 5'-TGA CAT ACA TCC CTC TCT G-3' in the presence of the FAM labelled wild-type beacon (5'-CGC GAT GAT TCT CAA CAG ATA CCC TCA CTT CAA TCG CG-3') and the HEX labelled mutant beacon (5'-CGC GAT ATT CTC AAC AGA CAC CCT CAC TTC AAT CGC G-3'). The 25 µl reaction mixture contained 200 ng of genomic DNA, 10 mM Tris/HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 4 mM MgCl<sub>2</sub>, 0.25 mM dNTPs, 50 ng of each primer, 200 nM of each beacon, and 2.5 U Taq-DNA-polymerase. The PCR

conditions were three minutes at 95°C, then 40 cycles of 30 seconds at 95°C, 30 seconds at 59°C, and 30 seconds at 72°C. Fluorescent signals were measured at 59°C. Genotypes were assigned using the iCycler iQ Optical System, software version 3.1. At each PCR run (in 96 well plates) sterile H<sub>2</sub>O instead of genomic DNA was added in several wells as a negative control for amplification. As the PCR-RFLP analyses were performed in the first half of 1999, only some of the samples were still available (125 of 149 controls and 149 of 151 cases) and these were re-evaluated by the iCycler method.

Genotype distribution of the EPHX1 Tyr113His polymorphism in patients with Crohn's disease and controls was now in HWE ( $\chi^2 = 2.47$ ,  $p = 0.12$  and  $\chi^2 = 0.82$ ,  $p = 0.37$ , respectively) and genotype distribution was not significantly different between cases and controls ( $\chi^2 = 3.5$ ,  $p = 0.17$ ). The Tyr allele frequencies of 0.70 and 0.68 obtained for cases and controls, respectively, were very similar to the corresponding values of 0.71 and 0.70, as reported by Cuthbert *et al.*

Thus in answer to the question as posed by Cuthbert *et al.*: "Genetic association between EPHX1 and Crohn's disease: population stratification, genotyping error, or random chance?", we can conclude that a genotyping error was responsible for our earlier published association between the EPHX1 Tyr113His polymorphism and Crohn's disease.<sup>1</sup> We now clearly state that our revised data do not support an association between this EPHX1 polymorphism and Crohn's disease. Similar genotyping errors may also be present in several other studies on the EPHX1 exon 3 polymorphism in association with a variety of diseases, as many studies were based on methods using a reverse primer covering the "119 silent mutation area" of the EPHX1 gene.<sup>3,6-9</sup> This may also have consequences for interpretation of results in the cited papers. However, a rapid literature search by Pubmed revealed more than 100 papers on EPHX1 polymorphisms over the past 10 years, suggesting that many more papers may deal with genotyping problems, as outlined above.

In addition, Cuthbert *et al.* also reported that another polymorphism tested in our study,<sup>1</sup> the CYP1A1 exon 7 Ile/Val polymorphism, was not in HWE in the control group. This is correct but this deviation from HWE may be attributed to random chance, due to the rarity of the Val allele in our population, which makes the  $\chi^2$  test inappropriate under such conditions. For instance, genotype distribution is in accordance with HWE when only two individuals less would have been classified as Val/Val homozygotes.

We thank Cuthbert *et al.* and Györfy and colleagues<sup>2</sup> for their interest in our work. In addition, we conclude that (interpretation of) data in many other published studies on the EPHX1 Tyr113His (exon 3) polymorphism should be critically re-evaluated.

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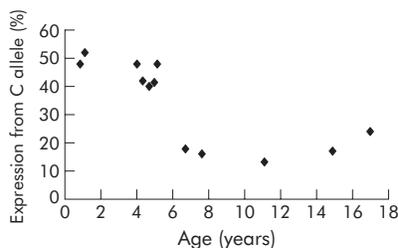
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### Transcriptional downregulation of the lactase (LCT) gene during childhood

Adult-type hypolactasia, characterised by bloating, gas formation, and diarrhoea after ingestion of lactose containing food, affects half of the world's population.<sup>3</sup> The molecular background of lactase non-persistence/persistence trait has been shown to associate with a single nucleotide polymorphism (SNP) C/T<sub>-13910</sub> residing 13910 base pairs upstream from the 5' end of the lactase (LCT) gene in an intron of the minichromosome maintenance 6 (MCM6) gene.<sup>2–4</sup> We have demonstrated a trimodal distribution of lactase activity in the intestinal mucosa in adults, with low lactase activity (4–9 U/g protein) in those with the C/C<sub>-13910</sub> genotype.<sup>3</sup> The C<sub>-13910</sub> and T<sub>-13910</sub> allele show differential regulation of lactase promoter activity and binding capacity for the nuclear proteins in electromobility shift assay.<sup>3–6</sup> Our recent analysis in a paediatric population demonstrated that the main time period for lactase downregulation in Finns and in Somalians is from five to 10 years of age.<sup>4</sup>

To further assess the role of the C<sub>-13910</sub> allele in downregulation of lactase activity during development, we isolated lactase RNA from intestinal biopsy samples with verified disaccharidase activities.<sup>7</sup> The study group comprised 15 subjects aged 10 months to 23 years, 12 with the C/T<sub>-13910</sub> genotype and three with the C/C<sub>-13910</sub> genotype. All subjects were heterozygous for the G/A<sub>+593</sub> polymorphism residing in exon 1 of the LCT gene. Relative expression of lactase mRNA transcribed from the C<sub>-13910</sub> and T<sub>-13910</sub> allele was assessed by quantitative minisequencing using the G/A<sub>+593</sub> polymorphism on the LCT gene as a marker. The methods used



**Figure 1** Relation of age to relative expression of lactase LCT mRNA from the C<sub>-13910</sub> compared with that from the T<sub>-13910</sub> allele. Actual relative expression of the C allele in the biopsy sample was obtained by relating the results of the minisequencing to an 11 point standard curve ( $y = 0.0135x - 0.9714$  up to 82%;  $y = 1 \times 10^{-5}e^{0.1129x}$  for 82–100%) obtained based on information of the relative amounts of G and A in a G/A<sub>+593</sub> heterozygous genomic DNA sample (for details of methods, see Kuokkanen et al<sup>7</sup>).

are described in detail by Kuokkanen and colleagues.<sup>3</sup> The study was approved by the ethics committee at the Helsinki University Central Hospital. All families gave their informed consent.

Subjects with the C/T<sub>-13910</sub> genotype (age range 10 months to 17 years) had high lactase activity, ranging from 21 to 113 U/g protein (mean activity 47 U/g protein; sample not available n = 2) except for one child presenting with low lactase activity (6 U/g protein). In this case the indication for endoscopy was exclusion of gastro-oesophageal reflux. Due to the very low lactase activity and the C/T<sub>-13910</sub> genotype, mutations underlying congenital lactase deficiency (CLD)<sup>8</sup> were screened for this patient and he was shown to be a carrier of a CLD mutation (unpublished data). Of the three subjects with the C/C<sub>-13910</sub> genotype, the oldest subject aged 23 years had low lactase activity (6 U/g protein) as expected; the five year old subject had high lactase activity (24 U/g protein). Lactase mRNA was transcribed in a 1:1 ratio from the C<sub>-13910</sub> and the T<sub>-13910</sub> allele in children younger than five years of age. In children over six years of age, relative lactase mRNA expression from the C<sub>-13910</sub> allele was reduced to 18% and 16% compared

with that from the T<sub>-13910</sub> allele (fig 1, table 1).

Our results show an increasing imbalance in relative mRNA expression levels of the C<sub>-13910</sub> and T<sub>-13910</sub> alleles in children aged >5 years. These results support the earlier findings on transcriptional regulation of the lactase gene<sup>9,10</sup> and the finding in our own laboratory that the persistent T<sub>-13910</sub> allele was shown to represent a mean of 92% of expressed lactase mRNA in C/T<sub>-13910</sub> heterozygous adults.<sup>3</sup> The decline in lactase mRNA expression transcribed from the C<sub>-13910</sub> allele in the intestinal mucosa occurs in parallel with the time period of the decline in lactase enzyme activity, indicating a causative role for the intronic region containing the C<sub>-13910</sub> allele. Characterisation of the transcriptional regulators at the C/T<sub>-13910</sub> enhancer element and the exact mechanism underlying C<sub>-13910</sub> allele specific downregulation of lactase activity awaits elucidation.

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**Table 1** Lactase activity, L/S ratio, and allelic ratio of the study subjects

Age (y)	C/T <sub>-13910</sub> genotype	Lactase activity (U/g protein)	L/S ratio	Allele ratio (%) C/T <sub>-13910</sub> *
0.8	CT	85	1.11	48/52
1.1	CT	113	1.02	52/48
4.0	CT	31	0.49	48/52
4.3	CT	53	0.48	42/58
4.7	CT	40	0.62	40/60
4.9	CT	–	–	42/59
5.1	CT	6	0.08	48/52†
6.7	CT	22	0.28	18/82
7.6	CT	–	–	16/84
11.1	CT	29	0.54	13/87
14.9	CT	21	0.40	17/83
17.0	CT	29	0.62	24/76
17.1	CC	–	–	52/48
5.0	CC	24	0.28	51/49
22.8	CC	6	0.08	49/51

\*Defined by assessing cSNP G/A<sub>+593</sub> in exon 1 of the lactase LCT gene.

†Carrier of a CLD mutation (unpublished data).

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## Cystic fibrosis transmembrane regulator gene carrier status is a risk factor for young onset pancreatic adenocarcinoma

Pancreatic adenocarcinoma is the fourth leading cause of cancer death in the USA. Although predominantly a cancer of the elderly, approximately 20% of patients are diagnosed under the age of 60 years. Younger patients are likely the best candidates for early surgical intervention, and patients at risk for young onset cancer comprise a logical focus for screening or prevention.

Carriers of mutations in the gene that encodes the cystic fibrosis transmembrane conductance regulator (*CFTR*) are associated with chronic idiopathic pancreatitis.<sup>1</sup> Chronic pancreatitis, in turn, increases the risk for pancreatic cancer by 26-fold.<sup>2</sup> Therefore, we hypothesised that mutations in *CFTR* may confer a higher risk of pancreatic cancer.

From October 2000 to April 2004, pancreatic cancer patients seen at the Mayo Clinic were ultra rapidly recruited to our study, with more than 75% of all such patients seen at the Mayo Clinic enrolled in the registry. This

**Table 1** Comparison of *CFTR* mutation frequencies detected in the young onset pancreatic cancer cohort versus the clinical database

	Young onset pancreatic cancer cases (<60 y old at diagnosis, n = 166)		Mayo Clinic clinical database reference group (n = 5349)	
	No	%	No	%
<i>CFTR</i> mutation non-carriers	152	91.6	5132	95.9
<i>CFTR</i> mutation carriers	14	8.4	217	4.1
Mutation distribution				
ΔF508	12	85.7	155	71.4
R177H	1	7.1	28	12.9
G551D			6	2.8
2789+5G>A			6	2.8
G542X			4	1.8
N1303K	1	7.1	3	1.4
1717-1G>T			2	0.9
3849+10kbC>T			2	0.9
A455E			2	0.9
R1162X			2	0.9
R347H			1	0.5
R553X			1	0.5
3905insT			1	0.5
621+1G>T			1	0.5
W1282X			1	0.5
1898+1G>A			1	0.5
R560T			1	0.5

Young onset pancreatic cancer cases were more frequent carriers of the *CFTR* mutations compared with patients in the control database (odds ratio 2.18 (95% confidence interval 1.24–3.29);  $p = 0.006$ ).

represents a substantial improvement over population based pancreatic cancer epidemiological studies, with participation rates ranging from 34.6%<sup>3</sup> to 45.6%.<sup>4</sup> Informed written consent and institutional review board approval were obtained.

As a pilot study, 33 patients were selected in whom a pathological diagnosis of pancreatitis was also noted at the time of pancreatic cancer surgery. The patients ranged in age from 41 to 81 years (median 65), and seven of the 33 had a diagnosis of pancreatitis made at least one year prior to cancer diagnosis. These patients were screened for variants in *CFTR* using the Tag-It Mutation Detection Kit, a clinically available kit testing for 40 mutations.

Of 33 samples tested, two patients (6%) were noted to have mutations in *CFTR*, both of which were the most common mutation identified in the *CFTR* gene, ΔF508. Both patients had young onset disease (ages 42 and 50 years). In total, seven patients in our pilot sample were below the age of 60 years, making the carrier rate 29% in this young onset subgroup.

Therefore, we designed a larger study to test the remainder of young onset cases in our registry, comprising a sequential unselected sample for mutations in *CFTR* (Cystic Fibrosis v3.0 ASR, Celera/Abbott), totalling 166 patients under the age of 60 years. Smoking status and family history were obtained from questionnaires. Personal history of chronic pancreatitis was identified by a single physician review of the medical records.

For a comparison group, the clinical database of *CFTR* analyses performed at the Mayo Clinic from November 2003 to May 2004 was utilised. Ethnic composition of cases and controls were highly comparable.

As shown in table 1, 14 of the 166 (8.4%) young onset pancreatic cancer cases were carriers for *CFTR* mutations, compared with 217 of 5349 (4.1%) patients in our control database ( $p = 0.006$ , odds ratio 2.18 (95% confidence interval 1.24–3.29)). There was no

significant difference in age of onset, pancreatitis, family history of pancreatic cancer, or smoking in carriers versus non-carriers of *CFTR* mutations.

Several cases of patients with cystic fibrosis (CF) and pancreatic adenocarcinoma have been reported,<sup>5–6</sup> and two cohort studies have shown an increased risk for pancreatic cancer among CF homozygotes.<sup>7–8</sup> Two studies have investigated *CFTR* mutation frequencies in pancreatic cancer patients, with negative results. However, both series only investigated one mutation (ΔF508), and neither focused on young onset patients.<sup>9–10</sup>

Our study represents the first positive association of pancreatic cancer risk with *CFTR* carrier status, with mutations conferring a twofold risk for cancer before the age of 60 years. The finding that only one of the *CFTR* carriers had an antecedent history of pancreatitis is intriguing, as either pancreatitis is subclinical or the presence of one mutant *CFTR* allele may increase the risk for pancreatic cancer through a mechanism independent of chronic pancreatitis. A larger study to confirm these results is ongoing.

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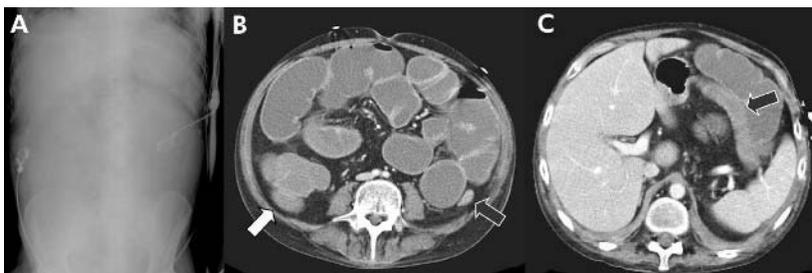
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## Distal intestinal obstruction syndrome in the early postoperative period after lung transplantation in a patient with cystic fibrosis: morphological findings on computed tomography

Distal intestinal obstruction syndrome (DIOS) occurs in 15.9% of adults with cystic fibrosis (CF).<sup>1</sup> Usually the diagnosis is based on history, physical examination, and plain abdominal roentgenogram. The increased risk of gastrointestinal complications such as DIOS is well known after lung transplantation.<sup>2–3</sup> Due to the added risk of gastrointestinal surgery in the postoperative period<sup>4</sup> and the generally good response to conservative treatment, it is necessary to distinguish DIOS from other gastrointestinal complications. Nevertheless, descriptions of computed tomographical patterns of DIOS in the international literature are rare.<sup>5</sup>

We present the case of a 34 year old male suffering from end stage CF. Because of gastrointestinal manifestations of CF, the patient had exocrine pancreas insufficiency. As a consequence of deterioration in respira-



**Figure 1** (A–C) Abdominal plain film showing remarkably little abdominal gas and poor delineation of the abdominal organs. Markedly distended small bowel loops and right hemicolon (white arrow) completely filled with a homogenous mass. Swelling of the intestinal wall with increased contrast medium enhancement. Thin transverse and descending colon with only few faeces (black arrows).

tory function, lung transplantation was performed. Despite enzymatic and propulsive medical treatment the patient developed an acute abdomen during the postoperative period. To determine the cause of his symptoms abdominal radiographs and computed tomography were performed. Abdominal plain films showed remarkably little abdominal gas and poor delineation of the abdominal organs (fig 1A). Contrast enhanced computed tomography showed massive dilatation of the small bowel and proximal colon with marked swelling of the intestinal wall (fig 1B, C). The lumen of the small intestine and proximal part of the ascending colon were filled with a homogeneous mass (fig 1B) with increasing roentgen opacity from the duodenum (approximately 19 Hounsfield units (HU)) to the right hemicolon (approximately 39 HU). Isolated air fluid levels were seen in the small bowel. The transverse, descending, and sigmoid colon were thin with only little faeces. There was no evidence of external compression. Based on these findings a diagnosis of DIOS was made. Laparotomy, performed due to failure of medical treatment, confirmed the diagnosis.

DIOS is unique to patients with cystic fibrosis. Intestinal obstruction developed due to accumulation of highly viscous mucofaeculent material in the terminal ileum and right hemicolon. Pancreatic insufficiency is a prerequisite for DIOS but is not its only pathophysiological cause. Other factors such as reduced intestinal water content, lower luminal acidity of the foregut, accumulation of intraluminal macromolecules, dehydration of the mucus layer due to altered intestinal secretion, and slow intestinal transit contribute to the development of DIOS.<sup>1–6</sup>

Plain films are of limited value in differentiating DIOS from other causes of acute abdomen. In the case of DIOS, they usually show typical signs of a small bowel ileus but other frequent reasons for ileus in patients with CF (for example, adhesions,<sup>3</sup> intussusception,<sup>7</sup> paralytic ileus due to perforated appendicitis,<sup>8</sup> or Crohn's disease<sup>9</sup>) cannot be excluded without further investigation. In our case, abdominal plain films showed no typical signs of small bowel ileus but little abdominal gas with poor delineation of the abdominal organs leading to the differential diagnoses of ascites, colitis, mesenteric infarction, and proximal bowel obstruction. In contrast with the plain abdominal radiograph, computed tomography showed the criteria of DIOS. The small bowel was completely filled

with a homogenous mass with increasing roentgen opacity from the duodenum (approximately 19 HU) to the right hemicolon (approximately 39 HU), suggesting increasing viscosity of the intestinal content due to water absorption. In accordance with previous descriptions of DIOS,<sup>3</sup> obstruction occurred in the right hemicolon.

Our case showed that abdominal plain films, as used in previous studies, are not adequate for the diagnosis of DIOS. Computed tomography can reveal the characteristic signs of DIOS and exclude inherent differential diagnoses. We have demonstrated for the first time that DIOS causes increasing opacity of intestinal contents during small intestinal passage, suggesting increasing viscosity.

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### Association of a new cationic trypsinogen gene mutation (V39A) with chronic pancreatitis in an Italian family

Predisposition to hereditary pancreatitis has been associated with mutations in three genes: protease, serine, 1 (PRSS1), which codes for cationic trypsinogen,<sup>1</sup> cystic fibrosis transmembrane conductance regulator (CFTR),<sup>2</sup> and serine protease inhibitor Kazal type 1 (SPINK1).<sup>3</sup>

We have identified a novel PRSS1 mutation in seven subjects with chronic pancreatitis (CP) from three generations of an Italian family. The index patient was a 57 year old man with CP referred to our hospital for ductal adenocarcinoma of the pancreatic head. Eleven relatives were examined, and an uncle, also with CP, had died in an accident.

Congenital malformations and alcoholic, biliary, obstructive, and autoimmune pancreatitis were ruled out. Eleven subjects gave their written consent to the study.

The cystic fibrosis assay (CF-OLA; Applied Biosystems, California, USA) was used to look for 31 frequent CFTR mutations in all subjects. The five exons of the PRSS1 gene were sequenced with the oligonucleotides described by Nishimori and colleagues.<sup>4</sup> The four SPINK1 exons were investigated by

denaturing gradient gel electrophoresis (DGGE). No CFTR or SPINK1 mutations were found although subject III-8 (with CP) carried the N1303K mutation in heterozygosis in the cystic fibrosis gene.

The PRSS1 exon 2 sequence of the index patient revealed a T>C change at nucleotide 116 (c.116 T>C) causing a valine to alanine substitution at codon 39 (V39A). This mutation was present in another six subjects with CP, diagnosed from exocrine insufficiency and computer tomography and magnetic resonance imaging demonstrations of typical ductal alterations and parenchymal calcifications. Two of these patients were also diabetic. In a further two patients, the genetic analysis was not performed, but CP was confirmed by clinical and morphological findings. The remaining four subjects had a normal pancreas and did not carry the V39A mutation (fig 1).

The lod score calculated for the association between V39A and CP was  $z = 3.0$  at  $\theta = 0.0$ . This mutation was not found in a DGGE investigation of 130 patients with sporadic CP.

Mean age of the patients was 47.22 ( $\pm 13.64$ ) years (median 54 (range 25-60)). Mean age at onset was 30.0 ( $\pm 7.35$ ) years (median 32 (range 19-40)) whereas in patients displaying other PRSS1 mutations, onset was typically during childhood or adolescence.<sup>5,6</sup>

An acute attack requiring hospitalisation formed the clinical overtone in six of the nine CP patients. The other three (III-4, III-5 and IV-2) presented morphological and functional evidence of CP at the time of the study but were asymptomatic. It is clear therefore that damage to the pancreas may occur prior to the clinical onset of CP.

In hereditary CP, the mechanism of the R122H mutation has been elucidated.<sup>1</sup> This substitution removes a hydrolysis start site and makes both trypsin and trypsinogen autolysis resistant. A similar mechanism has been proposed for the N29I mutation which alters protein conformation and masks the R122 site.<sup>7</sup>

Valine 39 is evolutionarily conserved in the trypsinogen gene of all terrestrial vertebrates<sup>8</sup> and would thus seem of importance in the protein's structure and function. As V39 is only 10 amino acids distant from N29, its replacement by alanine may result in abnormal conformation of the peptide and mask arginine 122 against enzymatic degradation. Further work is needed to define the mechanism and confirm this interpretation.

In conclusion, the presence of the V39A mutation in seven of the CP patients, its absence in their healthy relatives, the 3.0 lod score, and the strong evolutionary conservation of V39, all indicate that the novel mutation is the cause of CP in this family.

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**C Arduino**

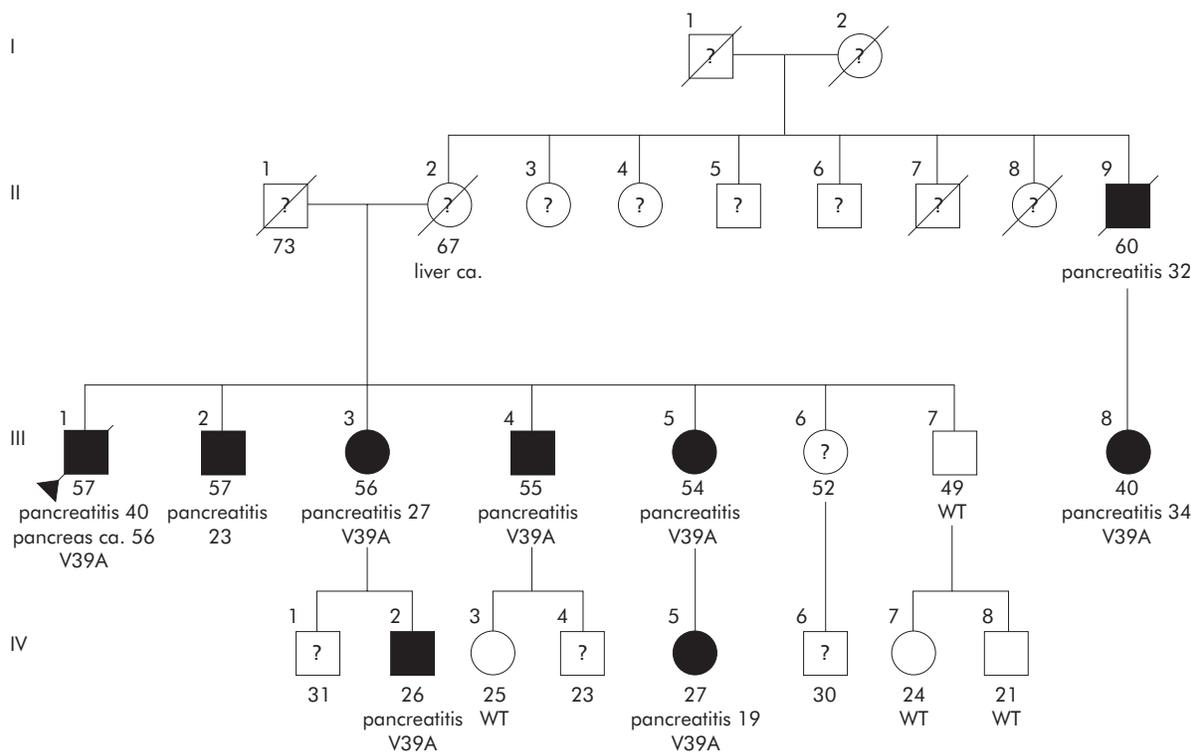
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**Figure 1** Pedigree showing the age of subjects, and for those with pancreatitis (black symbols) their age at onset (where known). WT, wild-type (that is, subjects without pancreatitis and without the V39A mutation); black triangle, index patient; ?, no clinical or genetic data available.

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## ITPA genotyping is not predictive for the development of side effects in AZA treated inflammatory bowel disease patients

We read with interest the letter by Colombel *et al* on the non-predictive value of ITPA genotyping for the development of myelosuppression after azathioprine (AZA) treatment (*Gut* 2005;**54**:565).

The level of thiopurine methyltransferase (TPMT) activity is determined by a common genetic polymorphism.<sup>1</sup> It was shown that low TPMT activity is linked to a higher relative risk of development of myelosuppression after AZA treatment.<sup>2</sup> Testing for TPMT genotype before the start of AZA treatment is of limited clinical value as myelosuppression resulting from TPMT mutations occurs in less than one third of patients with myelosuppression.<sup>3</sup>

Polymorphisms in genes encoding inosine triphosphate pyrophosphatase (ITPase), another enzyme involved in metabolism of AZA, have also been suggested to be associated with the development of side effects in AZA treatment.<sup>4</sup> Colombel *et al* show that there was no difference in the frequency of ITPA polymorphisms in 41 patients who developed AZA related myelosuppression in comparison with a previously published control population.<sup>5</sup> Unfortunately, this still leaves the question of other side effects such as flu-like symptoms, rash, and pancreatitis unanswered. In addition to the TPMT genotype, we determined the 94C>A ITPA polymorphism. All (109) patients with inflammatory bowel disease who started AZA treatment from January 2003 onwards were included, and side effects were determined. There was a mean follow up time of 13 months (range 4–24). The frequency of side effects was compared with the frequency of side effects in AZA treated patients without any (ITPA or TPMT) polymorphism. Notably, for patients with a heterogeneous TPMT or ITPA polymorphism, no preventive adjustments of AZA dosing were made.

In a patient group of a total of 109 patients, we found 10 who had a TPMT polymorphism and 12 who had a 94 C>A ITPA polymorphism. Eighty eight patients had none of the studied polymorphisms in TPMT or ITPA genes. Of the 12 patients who had an ITPA heterozygous polymorphism only two had side effects (17%). One had a rash and the other had complaints of arthralgia. In patients without any of the investigated

polymorphisms, 34 of 88 (39%) had side effects (summarised in table 1). There was one patient, receiving a normal dose of AZA, who had both a TPMT\*3A and an ITPA 94 C>A heterozygous polymorphism. Interestingly, this patient did not develop any side effects.

Our data confirms the results of Colombel's research by showing that an ITPA heterozygous polymorphism is not associated with an increased risk for the development of leucopenia. Additionally, we also found that there was no increased risk for the development of other side effects.

No conclusions can be drawn for patients who are homozygous for the ITPA 94 C>A polymorphism as none was included either in our study or in Colombel's. Marinaki *et al* included three patients with a homozygous 94 C>A polymorphism for ITPA and all three had side effects.<sup>4</sup> Therefore, further research on the risk of developing side effects in homozygous 94 C>A ITPA patients is desirable.

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**Table 1** Side effects in 109 azathioprine treated inflammatory bowel disease patients related to their thiopurine methyltransferase (TPMT) and inosine triphosphate pyrophosphatase (ITPA) genotypes

Side effect	No polymorphisms (88 of 109)	TPMT polymorphisms (10/109)				ITPA polymorphisms (12 of 109)	
		*1/*3A	*1/*3C	*3A/*3A	*3B/*3B	WT/94C>A	94C>A/94C>A
None	54	5	0	0	0	10	0
Leucocytes <2×10 <sup>9</sup> /l	0	0	0	1	1	0	0
Leucocytes 2–4×10 <sup>9</sup> /l	9	1	0	0	0	0	0
Hepatotoxicity	5	0	0	0	0	0	0
Pancreatitis	5	1	0	0	0	0	0
Gastrointestinal	7	0	0	0	0	0	0
Other	8	0	1	0	0	2	0
Total	88	7	1	1	1	12	0

One patient was included in both the TPMT polymorphisms column and in the ITPA polymorphisms column as he was heterozygous for the TPMT\*3A polymorphism and heterozygous for the ITPA 94 C>A polymorphism. Side effects categorised as "other" included rash, renal function disorders, vertigo, myalgia, and arthralgia.

## Lack of serum antibodies to membrane bound carbonic anhydrase IV in patients with primary biliary cirrhosis

Nishimori *et al* have recently reported the presence of autoantibodies against carbonic anhydrase IV (anti-CA IV) in patients with autoimmune pancreatitis (*Gut* 2005;**54**:274–81). Furthermore, serum antibodies to CA II (anti-CA II) were observed in several autoimmune conditions. We have now investigated the presence of anti-CA IV and anti-CA II in a large series of sera from patients with primary biliary cirrhosis (PBC) and controls.

CA II is known to be expressed in the cytosol of various types of epithelial cells, including those lining bile ducts, renal tubules, and salivary ducts. For this reason, CA II was suggested as a common antigen in conditions characterised by an autoimmune aggression against epithelia. In autoimmune pancreatitis, serum anti-CA II are useful diagnostic tools while in PBC they were first detected by Gordon *et al* in 5/6 sera from patients with antimitochondrial antibody (AMA) negative PBC.<sup>1</sup> Subsequent studies however demonstrated prevalence rates as high as 46% in PBC sera but failed to confirm their specificity for AMA negative sera.<sup>2,3</sup> Interestingly, anti-CA II were also shown to inhibit enzyme activity.<sup>4</sup>

Apart from cytosolic CA II, the CA family also includes a highly active membrane bound enzyme that was coined CA IV.<sup>5</sup> Both CA II and CA IV are abundantly expressed in human bile duct epithelial cells. Interestingly, mainly due to the sequence homology between CA II and CA IV and CA IV localisation on cell membranes, Nishimori *et al* hypothesised that the exposed CA IV might be more immunogenic than cytosolic CA II.

Seventy sera from patients with PBC (60 AMA positive; all anti-hepatitis C virus negative; 63 women; mean age 60 (SD 10) years) who attended our tertiary referral centre were consecutively enrolled in the study. Control sera were obtained from 50 healthy subjects matched with patients for sex and age class ( $\leq 50$  v  $>50$  years). All sera were tested by immunoblotting for anti-CA IV and anti-CA II, as previously described.<sup>2</sup> Briefly, proteins were denaturated and separated (10 µg/lane) on a 1.5 mm sodium dodecyl sulphate-12% polyacrylamide gel. Proteins were then transferred onto nitrocellulose (pore size 0.45 µm) using a semi-dry transfer system. The nitrocellulose membrane was cut into 4 mm strips and, after blocking with 5% non-fat milk, all strips were incubated with serum samples diluted 1:100 and 1:200 for anti-CA IV and 1:100 for anti-CA II. Rabbit horseradish peroxidase conjugated antibodies against human immunoglobulins G, A, and M (Dako, Glostrup, Denmark) was diluted 1:1000 and used as secondary antibody. Peroxidase development was obtained with 0.05% 4-chloro-1-naphthol in Tris buffered saline containing 20% methanol and 0.05% H<sub>2</sub>O<sub>2</sub>. A rabbit polyclonal antihuman CA IV antiserum<sup>6</sup> was used as a positive control throughout the study. CA IV antigen and anti-CA IV antisera were provided by Dr William S Sly (St. Louis, Missouri, USA).

Results demonstrated no reactivity against CA IV in any of the PBC or healthy control sera. In contrast, similar to previous reports, anti-CA II antibodies were detected in 6/70 (9%) sera from patients with PBC but were absent in control sera.

In summary, we submit that the hypothesis that antibodies against the membrane bound CA IV may play a role in PBC should be rejected, based on our experimental data on a large series of sera. Our finding may be secondary to a different cellular expression of CA IV in the target organ (that is, pancreatic and bile ducts) but only specific tissue studies can provide these answers. At present, therefore, anti-CA IV should be regarded as specific to autoimmune pancreatitis and research should focus on better defining their possible role in this condition.

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## Association of achalasia and dental erosion

Dental erosion is the dissolution of enamel and dentine caused by organic or inorganic acids.<sup>1</sup> The source of acid is normally either dietary acids<sup>2</sup> or regurgitation of stomach juice<sup>3</sup> into the mouth. Enamel and dentine begin dissolution at a pH of approximately 5.5.<sup>4</sup> In achalasia, bacterial fermentation of food produces lactic acid, with a minimum pH of approximately 3.5,<sup>5</sup> which has the potential to demineralise teeth if it reaches the mouth. This study investigated whether regurgitated lactic acid fermented from food lying within an achalasic oesophagus causes dental erosion. The aim of the study was to measure the prevalence of dental erosion in patients referred for management of untreated achalasia and to compare the results with a control group.

Patients referred to the oesophageal laboratory from a variety of medical sources for investigation of achalasia were recruited. Manometry was used to diagnose the presence of achalasia in all subjects. Ethics approval was provided from the local hospital and each patient gave informed consent for assessment of erosion. The distribution and severity of dental erosion was determined using the Smith and Knight tooth wear index (TWI).<sup>6</sup> All tooth assessments were carried out by the first author under ideal conditions. The index scores

erosion on a five point scale, with 0 representing no erosion and a score of 4 representing pulpal exposure. A control group, without symptoms or history of gastro-oesophageal reflux disease, were selected from the partners of patients attending for oesophageal tests. Inclusion criteria did not take into account the presence or absence of dental erosion. A dietary questionnaire was used to exclude subjects with a high intake of dietary acids. Mann-Whitney U tests were used to compare patients with controls for differences in tooth wear scores. Intraclass correlation showed good agreement for the erosion scores (0.99).

Fifteen patients with achalasia (six males and nine females) with a mean age of 49 years (SD 18.4) were recruited over a two year period and compared with 32 controls (14 males and 18 females) with a mean age of 43 years (SD 16.8). Median percentage of teeth scoring a TWI of 2 or above was 21.4% (interquartile range (IQR) 11.46–30.77) in patients and 7.76% (IQR 0–12.2) in controls, for all tooth surfaces ( $p=0.001$ ). At the moderate level (score 3 and above), with dentine exposed for more than one third of the surface, patients had a median of 0% (IQR 0–16.1) and controls a median of 0% (IQR 0–0;  $p<0.001$ ). The distribution of the erosion was predominantly on the palatal surfaces of the upper incisors.

Achalasia is an uncommon disorder of the oesophagus in which there is failure of normal peristalsis in the body of the oesophagus and the lower oesophageal sphincter fails to relax.<sup>7,8</sup> The control group were recruited from the partners of the patients attending for oesophageal tests. Unfortunately, it was not feasible to undertake manometry in the controls as this was ethically unacceptable but there remains a possibility that some had asymptomatic reflux but not achalasia. If any controls had asymptomatic reflux they were at more risk of developing dental erosion but the results from the erosion scores seemed not to indicate this.

Ineffective oesophageal motility causes delayed acid clearance and its association with the presence of palatal dental erosion was reported by Bartlett and colleagues.<sup>9,10</sup> The results of this study support the hypothesis that oesophageal motility disorder has an important role in the development of dental erosion, albeit an extreme example. In this case, an obstructive oesophagus causes food fermentation, and in turn regurgitation of fermented food causes dental erosion. The presence of palatal dental erosion in patients with achalasia strongly suggests that the source of the acid within the oesophagus is lactic acid unlike reflux disease where hydrochloric acid from the stomach is responsible.<sup>11</sup> This study shows that in patients with achalasia, particular attention to the condition of their teeth needs to be addressed. In conclusion, achalasia is related to palatal dental erosion and the cause of the erosion is fermented foods and not regurgitated gastric juice.

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## BOOK REVIEW

### New Techniques in Gastrointestinal Imaging

Edited by S Halligan, H M Fenlon. New York: Published by Marcel Dekker Inc, 2004, pp 431.

Many areas of radiology are rapidly developing new techniques to answer clinical

problems or devising ways of refining current imaging techniques. Gastrointestinal imaging is no exception.

*New Techniques in Gastrointestinal Imaging* has been edited and written by experts in the field from the international community and encompasses the more recent developments in all aspects of gastrointestinal imaging. The book has been divided into chapters that either concentrate on a particular imaging technique (for example, computed tomography (CT) colonography) or those that cover recent developments in the investigation of a particular area (for example, the rectum). There are very comprehensive chapters covering the new CT and magnetic resonance (MR) techniques available for imaging the colon and small bowel. New CT and MR techniques for hepatic imaging are also included, with special reference to the development of CT angiography. There are excellent chapters on the use of microbubbles in ultrasound (US) and endoscopic US, both of which are good introductions to these techniques for those with limited previous knowledge or experience. Also included is a very useful chapter on positron emission tomography (PET) with a gentle introduction to the physics of the technique and current applications and limitations. New interventional imaging techniques are also covered, with chapters on radiofrequency ablation of liver lesions and on self expanding metallic stents in the colon.

I was however dismayed to find a section on defecating proctography, a technique I had rather hoped had been consigned to history. The current method seems to have changed little from my days as a junior registrar banished to the barium room although new MR techniques are described.

This book has been written to update the general radiologist in areas of gastrointestinal radiology that have changed significantly in recent times. This it does very well, with concise descriptions of the techniques, thorough discussions on clinical use, and handy tips on image interpretation. As such, there are chapters in the book that need some

background knowledge of radiological techniques to appreciate the new developments (for example, CT and MR chapters on liver imaging). However, all chapters provide a good setting for each of the new techniques so that the interested gastroenterologist would find useful information on the current role of each investigation, its performance with relation to more established techniques, and future developments.

A Graham

## CORRECTIONS

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In the Editor's quiz: GI snapshot on p1272 of the September issue (D Joshi, J Dunga, A James and MM Yaqoob. An unusual case of hepatosplenomegaly. *Gut* 2005;**54**:1272; doi:10.1136/gut.2005.064824) the second author's name should read Dungu not Dunga.

In the Gut Tutorial on p296 of the February issue the author's name and affiliation was omitted. The details are as follows: Robin Spiller, Professor of Gastroenterology, Wolfson Digestive Diseases Centre, University Hospital, Nottingham NG7 2UH, UK.

In the Gut Tutorial on p555 of the May issue the author names and affiliations were omitted from the original publication. This has been updated on the Gut website. The authors and affiliations are as follows:

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