Plexiform neurofibroma mimicking a pancreatic cystic tumour

Pancreatic neurogenic tumours are extremely rare. Among benign neurogenic tumours, schwannoma is more frequently encountered. 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 underlying lesions.

A bright appearance on T2 weighted magnetic resonance images is a characteristic of PNF. Secondly, surgical
resection was necessary to exclude malignancy which is more frequently encountered in PNF compared with classical neurofibromas. In addition to classical benign features, similar to published data on benign PNF, in PNF compared with classical neurofibromatosis which is more frequently encountered in NF1 syndrome, the absence of detectable genetic abnormalities and other clinical NF1 syndrome associated lesions in the present case could be explained by such a mechanism. 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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References


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.1 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 331 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.2 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.3

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 silent mutation area” of the EPHX1 gene.1-3 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, the CPY1A1 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 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 colleagues4 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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Conflict of interest: None declared.
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. The molecular background of lactase non-persistence/persistence trait has been shown to associate with a single nucleotide polymorphism (SNP) C/T_{13910} residing 13910 base pairs upstream from the 5’ end of the lactase (LCT) gene an intron of the minichromosome maintenance (MCM6) gene. 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_{13910} genotype. The C_{13910} and T_{13910} allele show differential regulation of lactase promoter activity and binding capacity for the nuclear proteins in electromobility shift assays. Our recent analysis in a paediatric population demonstrated that the main time period for lactase downregulation in Finns and in Somalis is from five to 10 years of age.

To further assess the role of the C_{13910} allele in downregulation of lactase activity during development, we isolated lactase RNA from intestinal biopsy samples with verified disaccharidase activities. The study group comprised 15 subjects aged 10 months to 23 years, 12 with the C/T_{13910} genotype and three with the C/C_{13910} genotype. All subjects were heterozygous for the G/A_{593} polymorphism residing in exon 1 of the LCT gene. Relative expression of lactase mRNA transcribed from the C_{13910} and T_{13910} allele was assessed by quantitative minisequencing using the G/A_{593} polymorphism on the LCT gene as a marker. The methods used are described in detail by Kuokkanen and colleagues. 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_{13910} 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 = 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-esophageal reflux. Due to the very low lactase activity and the C/T_{13910} genotype, mutations underlying congenital lactase deficiency (CLD) 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_{13910} 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_{13910} and the T_{13910} allele in children younger than five years of age. In children over six years of age, relative lactase mRNA expression from the C_{13910} allele was reduced to 18% and 16% compared with that from the T_{13910} allele (fig 1, table 1).

Our results show an increasing imbalance in relative mRNA expression with the C_{13910} and T_{13910} alleles in children aged >5 years. These results support the earlier findings on transcriptional regulation of the lactase gene and the finding in our own laboratory that the persistent T_{13910} allele was shown to represent a mean of 92% of expressed lactase mRNA in C/T_{13910} heterozygous adults. The decline in lactase mRNA expression transcribed from the C_{13910} 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_{13910} allele. Characterisation of the transcriptional regulators at the C/T_{13910} enhancer element, and the exact mechanism underlying C_{13910} allele specific downregulation of lactase activity awaits elucidation.

Acknowledgements

We are grateful to the children and their families for their participation. Mrs Sari Näsman and Mervi Mansikkamäki at the Day Surgery Unit, Hospital for Children and Adolescents, are acknowledged for coordinating and managing the sample collection. Funding was provided by the Sigrid Jusélius Foundation, Helsinki, Finland, the Helsinki University Hospital Research Funding, Helsinki, Finland, the Finnish Cultural Foundation, the Maud Kuistila Foundation, and The Research Foundation of Orion Pharma, Espoo, Finland.

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

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>C/T_{13910} genotype</th>
<th>Lactase activity (U/g protein)</th>
<th>L/S ratio</th>
<th>Allele (%)</th>
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</thead>
<tbody>
<tr>
<td>0.8</td>
<td>CT</td>
<td>85</td>
<td>1.11</td>
<td>48/52</td>
</tr>
<tr>
<td>1.1</td>
<td>CT</td>
<td>113</td>
<td>1.02</td>
<td>52/48</td>
</tr>
<tr>
<td>4.0</td>
<td>CT</td>
<td>31</td>
<td>0.49</td>
<td>48/52</td>
</tr>
<tr>
<td>4.3</td>
<td>CT</td>
<td>53</td>
<td>0.48</td>
<td>42/58</td>
</tr>
<tr>
<td>4.7</td>
<td>CT</td>
<td>40</td>
<td>0.62</td>
<td>40/60</td>
</tr>
<tr>
<td>4.9</td>
<td>CT</td>
<td>6</td>
<td>0.08</td>
<td>48/52</td>
</tr>
<tr>
<td>5.1</td>
<td>CT</td>
<td>6</td>
<td>0.08</td>
<td>48/52</td>
</tr>
<tr>
<td>6.7</td>
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<td>7.6</td>
<td>CT</td>
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<td>13/87</td>
</tr>
<tr>
<td>11.1</td>
<td>CT</td>
<td>21</td>
<td>0.40</td>
<td>17/83</td>
</tr>
<tr>
<td>14.9</td>
<td>CT</td>
<td>29</td>
<td>0.62</td>
<td>24/76</td>
</tr>
<tr>
<td>17.0</td>
<td>CT</td>
<td>29</td>
<td>0.62</td>
<td>24/76</td>
</tr>
<tr>
<td>17.1</td>
<td>CT</td>
<td>29</td>
<td>0.62</td>
<td>24/76</td>
</tr>
<tr>
<td>5.0</td>
<td>CT</td>
<td>24</td>
<td>0.28</td>
<td>51/49</td>
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<tr>
<td>22.8</td>
<td>CT</td>
<td>6</td>
<td>0.08</td>
<td>49/51</td>
</tr>
</tbody>
</table>

*Defined by assessing cSNP G/A_{593} in exon 1 of the lactase LCT gene.
†Carrier of a CLD mutation (unpublished data).
Conflict of interest: None declared.

Wang Y

conductance regulator (encodes the cystic fibrosis transmembrane early surgical intervention, and patients at

Although predominantly a cancer of the

leading cause of cancer death in the USA.

regulator gene carrier status is a

Cystic fibrosis transmembrane

Pancreatic adenocarcinoma is the fourth

leaving cause of cancer death in the USA.

Altogether 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

codes the cystic fibrosis transmembrane conducance regulator (CFTR) are associated with chronic idiopathic pancreatitis. Chronic

pancreatitis, in turn, increases the risk for pancreatic cancer by 26-fold. Therefore, we

also propose 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

represents a substantial improvement over

population based pancreatic cancer epidemiological studies, with participation rates ranging from 34.6% to 45.6%. 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 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

taq-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, AF508. 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 unsel-

ccted 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 his-
tory 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, pan-

creatitis, 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, and two cohort studies have shown an increased risk for pancreatic cancer among CF homozygotes. Two studies have investigated CFTR mutation frequencies in pancreatic cancer patients, with negative results. However, both series only investi-
gated one mutation (AF508), and neither focused on young onset patients.

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.

Acknowledgements

We thank the patients in this study and the contributions of Tammy Dahl, RN, Kathy Liffrig, Cynthia Nixa, Diane Batzel, Que Luu, Suresh Chari, MD, and Thomas Smyrk, MD.

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

<table>
<thead>
<tr>
<th>Young onset pancreatic cancer cases (&lt; 60 y old at diagnosis, n = 166)</th>
<th>Mayo Clinic clinical database reference group (n = 5349)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFTR mutation non-carriers</td>
<td>CFTR mutation carriers</td>
</tr>
<tr>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Mutation distribution</td>
<td></td>
</tr>
<tr>
<td>AF508</td>
<td>12</td>
</tr>
<tr>
<td>R175H</td>
<td>1</td>
</tr>
<tr>
<td>G551D</td>
<td>6</td>
</tr>
<tr>
<td>2789+5G/A</td>
<td>6</td>
</tr>
<tr>
<td>G542X</td>
<td>4</td>
</tr>
<tr>
<td>N1303K</td>
<td>1</td>
</tr>
<tr>
<td>1717T/17T</td>
<td>0</td>
</tr>
<tr>
<td>3849+106C&gt;T</td>
<td>0</td>
</tr>
<tr>
<td>A455E</td>
<td>2</td>
</tr>
<tr>
<td>R162X</td>
<td>2</td>
</tr>
<tr>
<td>R347H</td>
<td>1</td>
</tr>
<tr>
<td>R553X</td>
<td>1</td>
</tr>
<tr>
<td>390T</td>
<td>1</td>
</tr>
<tr>
<td>621T/12T</td>
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</tr>
<tr>
<td>1129</td>
<td>0</td>
</tr>
<tr>
<td>2182X</td>
<td>0</td>
</tr>
<tr>
<td>1898G/1898G</td>
<td>0</td>
</tr>
<tr>
<td>R560T</td>
<td>0</td>
</tr>
</tbody>
</table>

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).
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). 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. As a consequence of deterioration in respiratory 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 Hoursfield units (HU)) to the right hemilocolon (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 mucous-faculent material in the terminal ileum and right hemilocolon. Pancreatic insufficiency is a prerequisite for DIOS but is not its only pathophysiologic 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 motility and microvascular insufficiency of the colon, 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.

Plain films are only 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, intussusception, paralytic ileus due to perforated appendicitis, or Crohn’s disease) 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 infarcation, 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 hemilocolon (approximately 39 HU), suggesting increasing viscosity of the intestinal content due to water absorption. In accordance with previous descriptions of DIOS, obstruction occurred in the right hemilocolon.

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.

References
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, cystic fibrosis transmembrane conductance regulator (CFTR), and serine protease inhibitor Kazal type 1 (SPINK1).

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. The four SPINK1 exons were investigated by denaturant 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 θ = 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 ± 7.35 years (median 32 (range 19–60)). Mean age at onset was 30.0 ± 13.64 years (median 25 (range 25–60)). In conclusion, the presence of the V39A mutation in seven of the CP patients, its absence in their healthy relatives, and asymptomatic family members, indicates that the novel mutation is the cause of CP in this family.

Acknowledgements

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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.
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 genetically polymorphism. It was shown that low TPMT activity is linked to a higher relative risk of development of myelosuppression after AZA treatment. Testig 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.

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. 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 cohort population. 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 heterogenous 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 94C>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 TPMT3A 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. Therefore, further research on the risk of developing side effects in homozygous 94 C>A ITPA patients is desirable.

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

<table>
<thead>
<tr>
<th>Side effect</th>
<th>No polymorphisms (88 of 109)</th>
<th>TPMT polymorphisms (10/109)</th>
<th>ITPA polymorphisms (12 of 109)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/3A</td>
<td>*1/3C</td>
</tr>
<tr>
<td>None</td>
<td>54</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Leucocytopenia &lt;2 x 10^9/l</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Leucocytopenia 2-4 x 10^9/l</td>
<td>9</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hepatotoxicity</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>8</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>88</td>
<td>7</td>
<td>1</td>
</tr>
</tbody>
</table>

One patient was included in both the TPMT polymorphisms column and in the ITPA polymorphisms column as he was heterozygous for the TPMT3A 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 cytoplasm 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) positive; 5/6 PBC. Subsequent studies however demonstrated prevalence rates as high as 46% in PBC sera but failed to confirm their specificity for AMA negative sera.

Interestingly, anti-CA II were also shown to inhibit enzyme activity.

Apart from cytosolic CA II, the CA family also includes a highly active membrane bound enzyme that was coined CA IV. Both CA II and CA IV are abundantly expressed in human bile duct and 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 (<50 v >50 years). All sera were tested by immunoblotting for anti-CA IV and anti-CA II as previously described.

Briefly, proteins were denatured and separated (10 μg/lane) on a 15 mm sodium dodecyl sulphate-12% polyacrylamide gel. Proteins were then transferred onto nitrocel-lulose (pore size 0.45 mm) 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 1:1000 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% H2O2. A rabbit polyclonal antihuman CA IV antisera was used as a positive control throughout the study. CA IV 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, and that CA IV is not a target autoantigen in 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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References

Association of achalasia and dental erosion

Dental erosion is the dissolution of enamel and dentine caused by the action of organic acids.1 The source of acid is normally either dietary or from fermentation of food within the oesophagus. In achalasia, regurgitation of stomach fluid rich in lactic acid is common.2,3 Furthermore, bacterial fermentation of food produces lactic acid in the achalasic oesophagus.4 Erosion is a multifactorial disease.5,6 The aim of the study was to measure the prevalence of dental erosion in patients referred for management of upper oesophageal 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. Patients referred to the dental clinic were excluded. After ethics approval was obtained from the local hospital and each patient and healthy volunteer 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).7 All tooth assessments were carried out by the first author under ideal conditions. The index scores were calculated 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 determine the patients’ diet 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, the 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 a common 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.8,9 The control group were recruited from 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.10 The result of this study supports the hypothesis that oesophageal motility disorders have 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 regurgitated fermented food causes dental erosion. The prevalence 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.11 This study shows that in patients with achalasia, particular attention to the condition of their teeth needs to be addressed. In conclusion, achalasia is not related to palatal dental erosion and the cause of the erosion is fermented foods and not regurgitated gastric juice.

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Conflict of interest: None declared.

References

BOOK REVIEW

New Techniques in Gastrointestinal Imaging

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 Dunga 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: S A Khan, A Miras, Liver Unit, Department of Medicine A, Faculty of Medicine, Imperial College London, St Mary’s Hospital Campus, South Wharf Road, London W2 INY, UK; M Pelling, Department of Radiology, Faculty of Medicine, Imperial College London; S D Taylor-Robinson, Liver Unit, Department of Medicine A, Faculty of Medicine, Imperial College London.